DEFICIENCY DISEASE

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Functional and Structural Changes in Mammalia Which Result from Exogenous or Endogenous Lack of One or More Essential Nutrients

By

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To
Richard H. Follis, Sr., M.D.
Arnold R. Rich, M.D.
Edwards A. Park, M.D.

PREFACE

This volume is the outgrowth of a monograph, *The Pathology of Nutritional Disease*, which was published early in 1948 The cordial reception which it received has prompted a desire to bring the material up to date, since that manuscript went to the press over ten years ago But of even more consequence was a growing feeling of dissatisfaction in the way I had presented the subject of nutritional disease That volume mirrored my own introduction to the field of nutrition during the exciting days of the late 1930's and early 1940's when I was privileged to collaborate with Drs E V McCollum, H G Day, E Orent-Keiles, and M. M Wintrobe. Studies of deficient states produced by a lack of single essential nutrients were then being emphasized, investigations of multiple deficiency states were not popular. As a result, I restricted the discussion to physiological, biochemical and anatomical changes in the deficient mammalian host which could be ascribed to a lack of a *single* nutrient Abnormal states which could be attributed to lack of *multiple* essentials were excluded Thus, *The Pathology of Nutritional Disease* dealt almost entirely with experimentally produced nutritional syndromes, this meant it was concerned in large part with nutritional disturbances in laboratory animals.

In the present volume I have attempted to remedy this situation by broadening the scope of the material which is to be presented In the first place, disturbances will be looked upon from two standpoints. (1) those resulting from deficiencies of *single* nutrients, usually dietary in origin and *experimentally produced, and (2) those resulting from lack of multiple* nutrients, which is the way most deficiency diseases occur naturally Secondly, we cannot consider only disturbances caused by the reduction in the dietary intake of a single nutrient or group of essentials. Our approach must be broadened so as to include disturbances ascribed to all of the conditioning factors which might produce general or local reductions or deficiencies of essential nutrients. Hence, I have chosen the title, *Deficiency Disease*, which I feel is more appropriate than *Nutritional Disease*, since the latter usually has a much wider meaning to include diseases produced by too much food and by metabolic disorders of varied natures. The scope of this monograph is therefore limited to those functional and anatomical changes in cells, tissues, or organs which may result from a lack of one

or more nutrients which such cells need. These alterations may be effected by impaired ingestion, assimilation, excretion, circulation, or metabolism. The distinction is not as fine as one might like, yet it allows one to restrict the material and perhaps lets him not be overly criticized when something is omitted. *I have tried to include all of the recognized naturally occurring human syndromes. Some are doubtless given more space than others, which* treatment mirrors my own experience.

Most of the functional and anatomical changes which are described herein are associated with a lack of exogenous essential nutrients, that is, those which must be supplied to the organism preformed in varying degrees of complexity During recent years we have become more and more aware of situations in which certain cells and tissues of the organism may suffer from a lack of some endogenous nutrient, that is, one which is ordinarily made from the exogenous essentials. A good example of *this situation is the hypoglycemic state, which may arise as a result of liver disease, hormonal imbalance, et cetera. Moreover, in this general sense* any genetic disease may be viewed as a fundamental expression of protein deficiency. At the present time sufficient data are not available with which to develop this phase of *Deficiency Disease* as completely as we should like However, the concept of "the biochemical lesion" which was developed by Peters over twenty years ago and his more recent hypothesis of "lethal synthesis" both point to an approach which will doubtless be worthy of further exposition at some future date.

The bibliography is a condensation of the publications which have been consulted. All could not be *included.* My apologies go to those who feel their material should have been cited. It is hoped that the references which are provided will aid in exploring a given subject further.

In preparing this monograph I have restudied much of the experimental material which has been reported previously. Many histological preparations have been rephotographed. Most of the microphotographs have not been published before For these I wish to thank Mr. Robert W. Nye of the *Photomicrography Section, Armed Forces Institute of Pathology.*

Numerous investigators have allowed me to reproduce illustrations, published and unpublished from their work My sincere appreciation goes to Drs. Maurice Sullivan, Paul Boyle, D T. Smith, D. W. Woolley, G. Goldsmith, G. H. Cartwright, J H Baxter, T. D. Spies, S. Ansbacher, J. Warkany, J. R M. Innes, R. W Vilter, W H Horwitt, A. Schaefer, C. Tejada, and H A. P. C Domen. Drs. E Lowenhaupt, K E. Mason, E H Oppenheimer, R. J. Lukes, J H. Yardley, J. N P Davies and A R Rich have allowed me to study and reproduce some of their material.

For the preparation of the manuscript I am indebted to Martha G.

Edson whose patience and diligence have contributed much to this entire undertaking

My relations with Mr Charles C Thomas and Mr. Payne E L. Thomas have been most cordial. It is a pleasure to express my appreciation for their help and continuing interest.

My scientific career has been sired by three, my late father, Richard H Follis, Sr, and two who have been more than step-fathers, Arnold R. Rich and Edwards A Park The dedication of this monograph allows me to pay tribute to each of them My father, a surgeon, early stimulated in me an interest in medicine and provided me with an opportunity to follow this career. First as a student, then as a collaborator with Arnold R Rich, I have gained more than words can ever express. His stimulation, constructive criticism, counsel and example have immeasurably influenced my own development Dr. Park introduced me to deficiency disease when we began our studies of disturbances in bone in 1937 In my friendship and collaboration with him I have been blessed in a way that only those who truly know him can appreciate

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PART I

DEFICIENCY DISEASE IN GENERAL

INTRODUCTION

In order to maintain the structural and functional integrity of its cells, the mammalian organism needs only a few of the 102 elements which are found in the periodic table Of these essentials, the three atoms carbon, hydrogen and oxygen make up water, simple lipids and carbohydrates If to these are added nitrogen and sulfur, the proteins and other nitrogenous compounds may be formed The atoms of calcium and phosphorus give stability to the skeleton Potassium, sodium, and chlorine maintain, in part, the electrolyte composition of tissue cells and circulating fluids Certain atoms are concerned with the activity of many enzyme systems or are integral parts of enzymes themselves Such include copper, iron, magnesium, manganese, zinc and molybdenum Cobalt is a structural part of vitamin B_{12} or cobalmin, while atoms of iodine are incorporated into the active principle of the thyroid gland.

This monograph is concerned with physiological and morphological alterations which occur in cells and tissues when they become deficient in one or more essential nutrients Deficiency disease syndromes may be produced at will in the experimental animal by causing a single nutrient to be lacking in the diet Such conditions are encountered infrequently, however, in naturally occurring nutritional diseases of animals and man. Here multiple deficiencies exist, so that the problem becomes much more complex Unfortunately, save for certain studies carried out during the early days of nutritional research, few experimental investigations on multiple deficiency states have been conducted Studies of this group are of great importance to elucidate those multiple deficiency disease syndromes which occur naturally in animals and man

Before considering certain broad aspects of deficiency disease, it might be useful to review briefly the chemical composition of the organism, since in the pages which follow we shall be dealing with its various constituents, at least many of which it cannot manufacture

As is well known, the intact organism consists in large part of water. Values approximating 70 per cent have been reported in the new-born infant,[1] the thirty-five year old "normal" male, who was analyzed by Mitchell *et al*,[2] contained 67 8 per cent water. The fat content of both the whole organism and its constituent parts is much more variable. For total lipid content the following values are representative· infant, 16 per cent, adult, 12 5 per cent. Values for various tissues will be found in Deuel's monograph.[3]

The total nitrogen content does not appear to vary much above or below 2 per cent[3]

Excluding carbon, hydrogen, oxygen, nitrogen and sulfur, certain of the elements are present in appreciable amounts. On a total weight basis such include potassium (.35 per cent), sodium (.15 per cent), magnesium (.05 per cent), chlorine (.15 per cent), calcium (2 0 per cent) and phosphorus (1 1 per cent).[4] Approximate values for potassium and sodium are shown in Table I.[5] Other elements are found in much smaller, or even trace amounts, among these are iron, copper, cobalt, zinc, manganese, iodine, fluorine, molybdenum, strontium, rubidium, lead, silicon, aluminum, bromine, and others which will be further considered on page 20.

It must be made clear now that there are several classes of materials which are found in the body. These may conveniently be grouped as follows *I* Those which are indispensable and which the organism cannot

TABLE I

POTASSIUM AND SODIUM CONTENT (mEq) OF TISSUES OF MAN[5]

	Weight in Kg.	*Potassium*	*Sodium*
Skeletal muscle	30 0	2730	810
Skin	18 0	360	1600
Red blood cells	2 4	252	36
Plasma	2 6	12	363
Bone	12 0	218	1600
Brain	1 9	150	133
Liver	1 8	135	74
Heart	0 3	24	11
Kidneys	0 3	18	22
Whole body	70 0	3900	4600

manufacture *de novo*. Such include the essential elements, certain amino acids, the vitamins and essential fatty acids *II*. Those which are indispensable but which the organism can form from those comprising Group *I* Examples are the non-essential amino acids, enzymes, hemoglobin, collagen, various carbohydrates, saturated and unsaturated fatty acids, et cetera. *III* Those which are dispensable and which appear to be adventitious as far as the organism is concerned. Such gain entrance via the food, water, external atmosphere, by contact, and so on They include a number of elements, as well as many more complex compounds

Now that the term "indispensable" has been used, upon what do we base the qualities, dispensable and indispensable? Since the early studies of F G. Hopkins, growth has been the criterion which was most commonly used to determine the indispensability or dispensability of a given nutrient. Such would indicate that young, i e., growing, animals have been employed most often, which is only natural since normal growth imposes the greatest

possible metabolic drain on the organism, and hence brings out most dramatically changes, which might occur slowly, or even not at all in the adult Reproductive ability is another quality which has been used to define indispensability. Other criteria are maintenance of nitrogen balance, absence of demonstrable metabolic defects, and failure to exhibit morphologic alterations. When a cell or tissue lacks one or more of the indispensable nutrients, any number of alterations, structural or functional, may be found. As far as the scope of this monograph is concerned, such changes comprise deficiency disease.

THE PATHOGENESIS OF DEFICIENCY DISEASE

In the laboratory, deficiency disease is most readily produced by employing a synthetic diet consisting of a protein such as casein or an amino acid mixture, fat including essential fatty acids, carbohydrate, salts, and appropriate amounts of all known vitamins The essential nutrient to be studied, whether it be an inorganic element, amino acid, vitamin, or fatty acid, is, of course, left out Such an experimental procedure may also be applied to man, though one does not always have to feed a highly purified diet, since foodstuffs of known composition can be utilized. Naturally occurring nutritional disease, such as is seen in man and animals, may result from a deficient or unbalanced intake as a result of economic factors, inherent deficiencies in foodstuffs themselves or imbalances. In addition, a number of subsidiary and contributory factors may lead to disease in man, or in animals, for that matter. These situations, which have been termed conditioned malnutrition,[7] deserve mention

A. Interferences With Intake: Loss of appetite (anorexia) due to a variety of causes may be responsible for a deficient intake of one or more nutrients Gastrointestinal disease such as peptic ulcer, biliary malfunction, or other metabolic disturbances, including pregnancy or food allergy, may be cited as causes of anorexia. Then, too, certain mechanical factors are of importance. Tumors within or without the intestinal tract may lead to partial or complete obstruction Lastly adentia, inflammation of the buccal tissues, et cetera, may interfere with the intake of foodstuffs Alcohol ingestion and chronic heart failure are other important factors

B. Interference With Absorption. Although adequate amounts of an essential nutrient may be ingested, optimal quantities may not be absorbed due to a variety of reasons Hypermotility of the intestinal tract may move the material through the lumen too rapidly for adequate absorption to take place. Insoluble complexes may form so as to prevent absorption of a particular material Familiar examples are the combination of certain metals

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be formed by the organism, are just as indispensable as those which have to be included in the diet. Such naturally include all carbohydrates, simple and complex, virtually all of the lipids and one-half of the amino acids. Hence, the health of cells in certain tissues may be affected adversely when excessive sugar is lost in the urine, when malfunction of the liver restricts plasma protein formation, or when heart failure leads to the restriction of the oxygen supply to the central cells of the liver lobule.

Bearing in mind then the importance of exogenous and endogenous foodstuffs and metabolites, what sort of a picture may we draw of the usual course of events which may be expected to occur as a deficient state develops? Although there are certain obvious exceptions which will be alluded to later, most workers[6] in the field of nutrition have adopted the hypothesis that the physiological and pathological changes which result from deficiencies of indispensable nutrients develop in a definite and orderly sequence: (1) Decreased concentration in the blood and intercellular fluids. (2) Decreased intracellular concentrations in one or more tissues. (3) Physiological changes in such tissues followed by (4) Pathological alterations which can first be seen microscopically, after which they become grossly visible (Figure 1)

It has been assumed that a decreased blood concentration of an essential nutrient is evidence of a decreased saturation of the body in that nutrient. Such a concept is, of course, based on "normal" or "lower limit of normal" values, which unfortunately have been extremely difficult to determine Chemical studies of blood plasma are therefore not entirely satisfactory, sometimes more useful than blood plasma levels are saturation or desaturation tests which lead one to the second link in the pathogenesis of deficiency disease· decreased concentrations of the nutrient in one or more tissues.

Here one is on firmer ground, since the actual concentration of a given nutrient can be measured during life in red blood cells and the white cell-platelet layer, as well as in liver and muscle biopsies Moreover, almost any tissue from an experimental animal or human can be studied chemically at autopsy. So, too, histochemical studies can be made of biopsy or autopsy sections, in this way decreases in nutrients such as vitamin A or ascorbic acid may be demonstrated under the microscope

When the concentration of a particular nutrient in a certain tissue falls to a critical level, one may expect evidences of metabolic derangements to appear. These manifest themselves in a variety of ways which are amenable to detection and measurement. Abnormal metabolites may be found in tissues, blood or excreta. Pyruvate, xanthurenic acid, and parahydroxyphenyllactic acid are examples of such abnormal materials. Certain liver function tests may be employed to detect changes in that organ. Then, too,

with phosphorus, or of oxalate, phytate and fat with calcium. *Absence of digestive secretions* may affect absorption; the efficacy of bile and pancreatic juice for the absorption of the fat-soluble vitamins is an excellent example. Certain essential nutrients may actually be destroyed or inactivated before absorption from the intestinal tract can take place. The destruction of thiamine by the enzyme, thiaminase, derived from certain fish, and the inactivation of biotin by avidin are examples of these complications. Finally, too vigorous therapy with mineral oil or cathartics must be cited.

C. Interference With Storage or Utilization: Even after adequate amounts of one or more nutrients are ingested and absorbed, they may be poorly stored or utilized. Hepatic disease, for instance, may lead to lowered concentrations of vitamin A in the liver; as a consequence the vitamin A level in the blood is diminished. So, too, when the thyroid gland is poisoned by thiouracil it is unable to utilize inorganic iodine to form physiologically-active organic forms.

D. Increased Excretion: Ingested materials may be absorbed normally, but re-excreted too rapidly to effect their necessary function. Such conditions may occur when polyuria due to a variety of causes is present Sweating is another example. Endocrine imbalance, such as hypoadrenalism with loss of sodium or the reverse loss of potassium, may have disastrous results Parathyroid imbalance promotes excessive loss of calcium and phosphorus from the organism Lastly, lactation is too often overlooked as a factor leading to a loss of one or more dietary essentials from the maternal organism.

E. Increased Requirements: Certain intakes of essential nutrients are adequate for the normal needs of the body, but occasionally, for a variety of causes, such needs are increased. Unless these requirements are met, the deficient state may develop. Fever, which results in an increased metabolism, is a prominent example. Hyperthyroidism is, of course, another fairly common case in point Pregnancy and excessive growth each require an excess of certain nutrients over the normal intake.

F. Inhibition By "Anti" Substances: Certain materials which are closely related in structure to vitamins and amino acids will block the action of these essential nutrients. For example when analogs of thiamine, nicotinic acid, riboflavin, pyridoxine, and pantothenic acid and of phenylalanine, are fed in the diet, evidences of deficiency in the specific material will appear

All of the factors just cited (A to F) presuppose an interference with the utilization of nutrients whose sources for the most part are exogenous. There are, as has already been noted, substances which, though they may

even microscopic lesions in every deficient state. If thiamine deficiency is produced in swine, for instance, an animal may die, having previously shown electrocardiographic abnormalities, at autopsy no microscopic changes may be found in the heart. The concept of the pathogenesis of dietary deficiencies, which is illustrated in Figure I, serves a useful purpose, especially in experimental studies of nutritional disease For fuller discussions from various standpoints references are available.[6, 8, 9, 10]

THE GENERAL EFFECTS OF INANITION

Anyone who studies the physiological and/or morphological consequences of deficiencies or single or multiple nutrients should realize that he may encounter either *nonspecific* or *specific* changes. When experimental animals or man are subjected to deficiency states, changes of a nonspecific nature may be found in any number of areas. In the main these alterations appear to be related to caloric deficiency and result from poor utilization of foodstuffs which follow the development of a given deficient state These effects have been widely studied and will only be touched on below. Anyone who plans to work in this field should orient himself by consulting Jackson's, *The Effects of Inanition and Malnutrition on Growth and Structure*[11] and *The Biology of Human Starvation* by Ancel Keys and his collaborators[12]

A much needed and pointed study has recently been reported on the rat by Widdowson and McCance.[13] Growing and adult animals were subjected to complete starvation and to caloric undernutrition During the absence of food young animals lost more weight than older ones. This was due to a loss of body fat. Starvation and undernutrition had no effect on hemoglobin levels in the adults, while the young subjected to caloric restriction showed only slight reductions No differences in loss of weight of the spleens, hearts and kidneys in proportion to body weight could be detected in the two groups In contrast, the livers of the young animals which were starved lost more weight than those animals whose food intake was restricted Glycogen had virtually disappeared from the livers of the starved animals while it was increased to 2.2 per cent in the restricted rats versus 0 9 per cent in the controls. This study shows some of the similarities and differences between starvation and undernutrition Histological observations on such animals would be of interest

At autopsy, the athreptic organism, whether it be rat or man, exhibits certain characteristic changes Some areas are affected earlier and more severely than others. In time, however, all tissues suffer One of the first and most conspicuous changes is a decrease in fatty tissue, not only in the

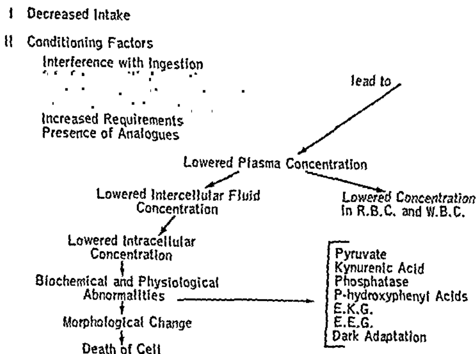


FIGURE 1. THE PATHOGENESIS OF DEFICIENCY DISEASE.

Schematic representation of factors which contribute to the various stages of deficiency disease See text for further explanation.

physiologic measurements of certain processes can be employed. electrocardiogram, electroencephalogram, tests of dark adaptation, and blood pressure determinations.

When the concentrations of a given nutrient have reached certain minimum tissue levels which are incompatible with life, morphologic alterations may be expected to occur. Such a situation however, does not necessarily mean that the entire organism dies. Quite the contrary, many obviously injured tissues may be examined microscopically before death. So, too, determination of the numbers and characteristics of the red blood cells and examination of the cornea by the low power of the slit lamp are other examples. In time, the gross changes occur and diagnosis may be made from macroscopic or clinical findings.

The above sequences, of course, do not take place in every instance, nor do they go on to completion. One need not necessarily find gross or

In partial or complete starvation there is atrophy of all internal organs to varying degrees [11] The brain usually shows least decrease in size. Sex organs, particularly the gonads, are strikingly affected.[16] The adrenal glands are usually enlarged.[17] The pituitary may show an increase in basophils and chromophiles and a decrease in acidophils [17] An extremely sensitive area is the growing skeleton [18, 19, 20] Cartilage cells and the osteoblasts respond nonspecifically by a slowing or even complete cessation of growth. Rarefaction of the bone is seen in the adult animal.

The literature is filled with studies of nutritional deficiencies in which certain nonspecific changes have been cited as resulting from a specific lack of a certain essential nutrient. Anyone who works in this field should be aware of this and should critically evaluate the changes which he may encounter.

How may one attempt to control the effects of inanition? It would appear that most investigators begin a study of nutritional disease by placing one group of animals on the deficient regimen and by having a second control group on the same diet to which has been added the missing nutrient. Both diets are then allowed *ad libitum* This technique may be improved, for, since the deficient animals do not usually eat as much as the controls, an attempt can be made to remedy this situation by giving the latter animals only the amount of food eaten by the deficient animals for some period before This is called the *paired feeding* technique However, such "paired-fed" controls usually grow more than the deficient animals because they are better able to utilize their restricted food intake Hence, a third method has been employed, though far too infrequently This pro-

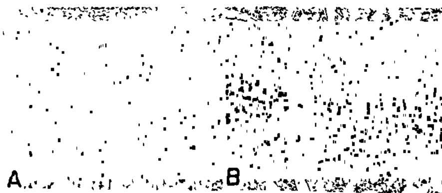


FIGURE 3 A GENERAL EFFECT OF INANITION

Cartilage and bone A Upper tibial epiphysis from normal growing rat B Same from rat whose caloric intake had been restricted Note difference in width of epiphyseal cartilage and diminution of bone in metaphysis of B H. and E (x 70).



FIGURE 2 [illegible]

Testis A Normal B Testis from rat whose caloric [illegible] *; difference in size of tubules and decrease in spermatogenesis in B.* [illegible] *e present in lumens of the tubules. Only Sertoli cells and a few spermatogonia remain.* H and E. (x 150)

subcutaneous areas but strikingly so in the mesentery, about the kidneys, uterus and testes, and in the retroperitoneal areas. Lymphoid tissue responds in a similar fashion. Hypoplasia of the lymph nodes, spleen, and thymus leads to great reduction in size of these structures The thymus is, with few exceptions, perhaps the best index of nutrition, particularly in the growing organism.[14]

Externally there may be a change in the coat with or without alopecia. Histologically the epidermis may show atrophy.[15] When alterations such as hyperkeratosis, parakeratosis, acanthosis and changes in the follicles or sebaceous glands are found, the dermal lesions are likely to be more specific in nature At this point it is important to emphasize that tissues and their cells respond in varying ways to injury. Those, like the skin, which have great powers of proliferation may exhibit this property. On the other hand, those such as the myocardium and neurons, which cannot regenerate, become atrophic or undergo necrosis. Inflammation and reparative phenomena may then be encountered.

MULTIPLE DEFICIENCY STATES

Early studies dealing with the nutritive value of single foodstuffs such as corn and rice clearly indicated that each was lacking in several important materials When corn was fed to swine and rats at least three factors were needed to supplement it[21, 22] These were protein, calcium and a fat-soluble material In addition, a fourth water-soluble factor was necessary to fortify polished rice[23] The importance of both of these studies is pertinent with respect to nutritional disease in corn and rice eating populations of the world today

With the advent of investigations dealing with the effects of deficiencies of single nutrients, the multiple deficiency approach came to be neglected. Though when deficiencies of several essential nutrients have been combined the resulting syndromes have been of interest For instance, if rats are made deficient in all of the B-group, except thiamine, skin lesions characteristic of pantothenic acid, pyridoxine, and riboflavin deficiencies do not develop, on the contrary, there is only atrophy of the epidermis and its appendages.[24] Again, when only thiamine and riboflavin are administered



FIGURE 5 A MULTIPLE DEFICIENCY STATE

Paws of rats which were placed on a synthetic diet containing only thiamine and riboflavin as members of the complex A Gangrene of the terminal phalanges of the first and second digits and almost complete amputation of the fourth B Localized gangrene of the first digit. (Courtesy of Dr Maurice Sullivan and *The Journal of Investigative Dermatology*[25])

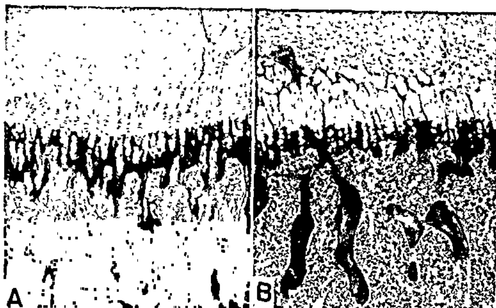


FIGURE 4. A GENERAL EFFECT OF INANITION.

Costochondral junction. *A* Costochondral junction from an eight year old child dying suddenly of cerebral hemorrhage Growth of cartilage appears normal though is not as active as it would be at an earlier age (see Figure 54). *B*. Costochondral junction from an eight year old child dying of chronic tuberculosis. Note decrease in width of proliferative cartilage cells and short stubby lattice of calcified cartilage matrix encased in bone H. and E. (x 50).

cedure restricts the food intake of the controls so that their weight gain will be the same as that of the deficient animals. This may be called the *paired weight gain* technique To our mind, particularly when tissue changes are being studied, this is the most critical method to employ. It is unfortunate that many nonspecific alterations have been designated as specific for a given nutrient by failure to compare animals by the *paired weight gain* procedure Hence, many experiments already reported will have to be repeated

Another point is important to realize, that is, when an acute, overwhelming deficiency state is produced, the morphologic changes may be slight or even absent in comparison with tissue alterations which may be encountered when the deficiency is more chronic and therefore not so severe. This situation is well exemplified in swine suffering from either acute or chronic thiamine deficiency The former animals may die, ostensibly of heart failure, with virtually no microscopic changes in the myocardium; while the latter usually exhibit extensive areas of damage in the cardiac musculature. Similar differences have been noted in many other deficient states.

Part II

The Inorganic Elements

as the B-group to rats, gangrene and spontaneous amputation of the digits may appear.[25] When, in another study, rats are made deficient in an element, potassium, and a vitamin, thiamine, which separately leads to myocardial necrosis, the hearts deficient in both together show no changes.[77] When a deficiency of sodium and chlorine is produced simultaneously, the results are different[104] from those which occur when either one or the other of the essential nutrients is withheld from the diet.[105, 115]

This approach would appear to be a most important one to pursue today, inasmuch as naturally occurring disease is usually based on multiple deficiency states. Intake of a dietary may be modified also by conditioning factors operating via the intestinal tract or kidneys. Hence the problem may become even more complex.

NUTRITIONAL IMBALANCE

The importance of dietary or metabolic imbalance is becoming more and more apparent. Such maladjustments as those of calcium and phosphorus, iron and phosphorus, fat and calcium, to name only a few, have been recognized for some time. Interrelations of certain nutrients one of which may be a precursor of another such as: methionine and cystine, methionine and choline, tryptophan and nicotinic acid, have further indicated the importance of balance of nutrients. So, too, the effects of protein, fat, or carbohydrate excess in the diet or an increased need for certain vitamins is clearly recognized.

A further fruitful approach appears to be the complex interrelationship of amino acids in the diet, which may be grouped under three general categories. imbalance, toxicity, and antagonism.[26] For instance, a supplement of the second most limiting amino acid in a protein may cause a more severe deficiency of the most limiting one. Such an effect may be seen as a deficiency in growth or as an increased deposition of fat in the liver. The feeding of an excessive amount of a single amino acid may be detrimental. Such may lead to the accumulation of harmful metabolic products. Finally, imbalance, which can better be regarded as antagonism, may be cited. Such is the leucine-isoleucine relationship in corn.

INTRODUCTION

That a certain group of elements is classed as indispensable has already been noted. In addition, even under specified normal conditions, other elements may, on chemical analysis, be shown to be present in the tissues of many mammalian organisms. Since no specific functions can be ascribed to these and since their absence does not impair the integrity of the organism, they must be regarded as dispensable. Some of the elements of this ubiquitous group will be discussed presently.

FUNCTIONS OF THE ESSENTIAL ELEMENTS

The essential group of elements plays a number of important roles in the organism for obvious reasons. We shall not specifically consider the four elements: hydrogen, carbon, oxygen, and nitrogen, which serve as structural components of tissues as parts of water, carbohydrate, protein and fat.

Calcium and phosphorus share a prominent place as structural components of bones and teeth. Here, they occur as crystals of hydroxyapatite and as such are discussed more fully on page 146.

Potassium, sodium, and chlorine are important in maintaining intracellular and extracellular electrolyte equilibrium.

Certain elements are of great importance in enzyme-catalyzed reactions.[27] Here they may activate the enzyme system and thus become fully as important a co-factor as are certain vitamins. Of the inorganic ions related to the various steps of carbohydrate metabolism, magnesium is found to be of pre-eminent importance. Manganese enjoys an important role in the reactions which make up the citric acid cycle. In both the glycolytic and Krebs cycles other elements such as potassium, calcium, cobalt and zinc may play important roles. Their exact mode of action is not too clear at this time. How they act to bring enzymes and substrates into an appropriate combination so that one can react with the other remains to be precisely shown.

Certain elements are an integral part of specific enzymes. For instance, zinc is present in carbonic anhydrase. Cobalt is a part of vitamin B_{12}, while iron is in the hemoglobin molecule, in catalase and peroxidase. So, too, copper is an integral part of tyrosinase, while molybdenum appears to be in the xanthine oxidase molecule.

In the pages which follow we shall point out from time to time the

PART II

THE INORGANIC ELEMENTS

Cesium has been demonstrated in the retina of the ox;[41] it is found in milk.[31] The function of cesium in the eye has not been elucidated When this element is substituted for potassium in a potassium-deficient diet, it partially protects the heart and kidneys from the effects of potassium deprivation [75]

Barium has been demonstrated in the eye of oxen; here it is present in the choroid in a concentration of 1.5 per cent of total dried tissue.[42]

Vanadium, which is found in milk[31] but not in the tissues of the newborn rat,[34] has been investigated with respect to its indispensability. If this element is necessary, it must be present in quantities less than 1-5 parts per million of diet, so that much more purified regimens will have to be concocted before the question of its indispensability can be settled.[43] It has been claimed that vanadium inhibits dental caries in the hamster [44]

Evidence that *selenium* must be added to the indispensable group has just been presented.[213] This element was found to occur in relatively large amounts in Factor 3, one of the materials which is effective in preventing massive hepatic necrosis in rats on a high yeast diet (page 101). Small amounts of selenium were found to be just as potent as Factor 3 itself This is an exciting finding, further information will be awaited with great interest

Certain other elements are also found in tissues or milk but have not been otherwise studied from the nutritional standpoint. These include *lead*,[45] *lithium*,[31] *strontium*,[31] *tin*,[45] and *titanium* [32] This brief discussion of the above group of elements, which is an incomplete listing, should give some idea of our knowledge of this subject. Undoubtedly, as more precise methods are developed, some of the elements just enumerated may be shown to be indispensable. The use of the hydroponic technique to grow foodstuffs in media uncontaminated by certain elements would seem to be worthy of application to this problem [259, 280] Moreover, the use of tissue cultures in assaying the inorganic requirements of mammalian cells would appear to be an important contribution to this field.[46]

INTERRELATIONS OF THE ELEMENTS

An interesting aspect of the study of the dispensable and indispensable elements in nutrition is that phase which deals with the substitution of one for another in physiological processes. A good deal is known of those elements comprising the alkali earth group in this regard but little information is available with respect to others For instance, sodium will partially replace potassium in the tissues of animals depleted in the latter element.[78] It is likely that sodium attempts to correct the acid-base balance of the cell, certain other functions of potassium, such as the maintenance of the integrity of heart muscle and kidney, do not appear to be affected, how-

effect of certain metals on enzyme systems, some of which can be measured quantitatively. This is an extremely important field for future exploration.

UBIQUITOUS ELEMENTS OF UNKNOWN FUNCTION

As was noted above there is a group of elements whose presence in the organism has been demonstrated by chemical methods of examination but whose indispensability has not yet been proven. Although a few of these elements may be shown to be essential at some later date, it is likely that most to be discussed are ingested with the food or inhaled into the lungs and are therefore purely fortuitous. The most important of these will be mentioned below; the data are based, for the most part, on spectrographic analyses of tissues and milk.

Since *boron* is essential for the growth of plants, this element has been investigated with respect to its indispensability for animals. Several independent investigations have failed to reveal any evidence that boron is an essential element for the rat; and the conclusion must be drawn that if boron is necessary for this species, the amount needed is less than .6 micrograms per rat per day.[28, 29, 30] Boron has been identified in milk[31] but not in the tissues of the newborn rat.[34] Based on growth studies in rats, it has been claimed that boron will replace potassium in a diet deficient in the latter element;[35] we have been unable to confirm this observation.[36]

Aluminum has been investigated in some detail. Traces are found in milk[31] and in the tissues of the newborn rat.[34] However, studies indicate that if this element is needed, extremely small quantities must be available, since the presence of 1 microgram in a milk diet employed in one investigation is sufficient to promote normal growth.[37]

Silicon is a fairly common constituent of most tissues[33, 34] and of milk.[31] No studies have been reported dealing with its indispensability for Mammalia.

Bromine is present in rather large quantities in the blood, urine and tissues.[38] Recent evidence has been presented which indicates that this element may be indispensable.[39] Further studies will be awaited with interest.

Arsenic is found in tissues and milk.[31, 33, 34] Two micrograms a day of this element are sufficient to supply the needs of growing rats,[40] rations lower than this in arsenic content have not yet been devised.

Rubidium is a common constituent of blood and tissues.[33] No studies have been reported in which a deficiency of this element has been produced. The present writer has shown that rubidium will partially substitute for potassium in a diet deficient in the latter element, since certain lesions which are characteristic of potassium deficiency fail to appear when rubidium is added to the diet.[75] The substitution is not a complete one.

ever. In fact, myocardial lesions resulting from potassium deficiency are made worse by excess sodium.[71] Two other elements of this group, rubidium and cesium, may more nearly replace potassium, at least for a short time.[75] When rubidium is added to a potassium-deficient diet, the characteristic necrosis of myocardial fibers and changes in the renal tubular epithelium fail to appear. The animals die, however. Cesium, which has a higher atomic weight than rubidium, only partially protects against cardiac and renal damage.

We cannot go into the many studies on enzyme reactions in which one element may fully or incompletely replace another, a subject which has received much attention from enzymologists.[27, 113]

THE ESSENTIAL ELEMENTS AND THE PERIODIC TABLE

In past years we and others appear to have speculated on the meaning, if any, of the distribution of the essential elements in the periodic table. At the moment, although several have written about this subject,[47, 48, 49] it would appear best to call attention to Figure 6 and let each speculate for himself.

IA	IIA	IIIB	IVB	VB	VIB	VIIB	VIII			IB	IIB	IIIA	IVA	VA	VIA	VIIA
H																
Li	Be											B	C	N	O	F
Na	Mg											Al	Si	P	S	Cl
K	Ca	Sc	Ti	V	Cr	Mn	Fe	Co	Ni	Cu	Zn	Ga	Ge	As	Se	Br
Rb	Sr	Y	Zr	Nb	Mo	Tc	Ru	Rh	Pd	Ag	Cd	In	Sn	Sb	Te	I

FIGURE 6. AN ABRIDGED PERIODIC TABLE OF THE ELEMENTS

The groups are arranged vertically, the periods, horizontally. The last periods have been omitted. The eighteen nutrients that are essential for *Mammalia* are shown in large type, others are in small type.

WATER

The inorganic elements as well as all organic metabolites must of necessity be transported in the blood plasma which is a liquid medium, find their way through the capillary walls into the interstitial fluid compartment, and then enter into the cells themselves. Since water is the vehicle

in vivo is not clear. A portion, but not all of the potassium leaving the cell, may be replaced by sodium ions. The cation content may be further restored by hydrogen ions[60] and by basic amino acids such as lysine and arginine.[61] Muscular contraction and activity of the nervous tissues are accompanied by a protoplasmic loss of potassium and by a gain in sodium concentrations[62]

Certain metabolic activities of cells are associated with the uptake of potassium The relation of this cation to carbohydrate metabolism has been recognized for some time[63] The disastrous effects of insulin therapy in acidotic, hyperglycemic diabetics, who do not receive potassium supplements, are noted elsewhere (page 290). Potassium is also of importance in the metabolism of protein. After animals have been depleted of protein, far less new nitrogenous materials are synthesized if potassium is not given during a repletion period Such a reciprocal relationship has been noted in animals[64] and in man.[65]

Other miscellaneous effects of potassium are its relationships to the activity of certain enzymes, for instance, that concerned with the phorphorylation of creatine[66] and with pyruvic phosphoferase.[67]

Potassium appears to be concerned with the secretion of acid by the cells of the gastric mucosa, at any rate, acidity is decreased when the organism is depleted of this important cation[68] Intestinal motility is also affected by decreased concentrations of intracellular potassium; decreased peristalsis may be followed by paralytic ileus.[69]

Morphological effects of potassium deficiency have been described in rats, mice, rabbits, dogs and calves The tissues which appear to suffer most are myocardium, striated muscle and kidneys

Employing diets containing only 0 01 per cent potassium and adequate in all other respects, the present writer in association with Orent-Keiles and McCollum[70] has described disturbances in growth, together with lesions of the heart and kidneys in rats Animals, acutely deficient, may die within a week, rats which have been maintained on a ration somewhat less deficient in potassium have been studied at varying intervals for almost a year

Grossly, after five to seven days on a potassium-low regimen, tiny gray opacities may be observed in the ventricles of the heart Microscopic studies of the myocardium reveal changes in such animals. The myocardial fibers at this time have lost their striations and appear hyaline and necrotic; coincident with these alterations the interstitial spaces are infiltrated by leukocytes The lesions range in extent from tiny foci, which early in the course of the deficiency involve only one or two muscle fibers, to large areas, as much as several low-power microscopic fields in greatest diameter, which develop as the deficiency progresses. In some hearts the tissues become

of ill-being vanished within a few hours of the restoration of fluid, and the symptoms of dehydration passed off long before physiological rehydration was complete."

During the four day period of dehydration no reduction in plasma volume was found. Serum sodium and chloride values rose while serum potassium concentration fell. Increased urea excretion was observed. These adult men lost over 3500 cc. of their body water (7 per cent body weight) but virtually none of the sodium from their extracellular fluids and only a little potassium from their intercellular compartment. The osmotic pressure of the organism rose, this may be related to the cause of death in dehydration.

POTASSIUM

The classic studies of Sydney Ringer in the 1880's clearly demonstrated the importance of potassium, sodium, and calcium for the *in vitro* function of the frog's heart.[50] These physiologic alterations have since been carried over to Mammalia. In studies so far reported potassium appears to effect its action via the extracellular environment. When concentrations of this cation are reduced so as to produce physiological alterations in the electrical activity of the myocardium, no reduction of intracellular potassium is demonstrable.[51]

The indispensability of potassium in the diet of the mammalian organism was first shown in 1918 by Osborne and Mendel.[52] Retardation in the growth of rats was found when the ration contained only .033 per cent of this element. It has since been shown that in the rat 0.17 per cent potassium appears to be the minimal amount which will support optimal growth.[53] This requirement is affected by the level of dietary sodium;[54] raising the latter necessitates less potassium.

Potassium comprises virtually all of the intracellular cations. Amounts vary from tissue to tissue. Normal values for man are noted on page 6, those for the rat may be found elsewhere.[55] The element has been demonstrated in tissue by a histochemical technique which employs cobaltinitrite.[56]

Ingested potassium is absorbed by the intestinal epithelial cells. Some is eliminated via the intestinal lumen; the rest is excreted by the kidneys. Here, two mechanisms appear to operate: glomerular filtration with tubular reabsorption and tubular secretion.[57] Adrenal cortical hormones have some control of potassium excretion.[58] The amount of potassium within the cells is related to its concentration in extracellular fluids. When the latter is reduced, the intracellular level falls,[59] though how long this may take

chloride, either in the diet or parenterally. Following the latter mode of administration death may occur suddenly. Microscopic examination reveals extensive necrosis of the myocardial fibers associated with little cellular reaction. Such lesions are far more extensive than those which we and others have observed in rats Their relation to the concomitant disturbance in protein metabolism needs to be elucidated. The relation of the sodium ion to the myocardial changes in potassium-deficient animals may help explain some of the morphologic variations which have been reported. Diets, which have been described in the literature, contain different potassium-sodium ratios. Cannon *et al.*[71] have raised the question as to whether the myocardial necroses result from potassium deficiency or sodium toxicity. This is an intriguing concept, warranting further study. Glycogen appears to accumulate in the myocardium of the potassium-depleted rat.[73]

The effect of exercise on the development of cardiac lesions has been studied by suspending weights to the thorax of deficient rats and having them swim until virtually exhausted. The alterations in the myocardium tended to be more extensive in those animals made to exercise than in de-

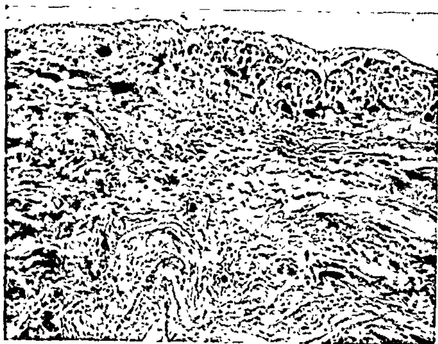


FIGURE 8. POTASSIUM DEFICIENCY.

Heart, rat. Myocardium from rat which had been on deficient diet for 327 days. Connective tissue has replaced myocardial fibers (x 150).

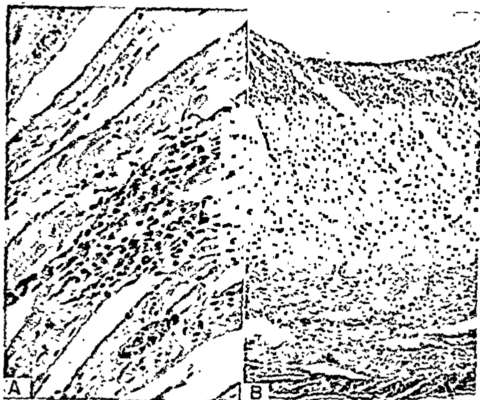


FIGURE 7. POTASSIUM DEFICIENCY.

Heart, rat. *A* Small focus of necrotic fibers in wall of left ventricle from rat on deficient diet for nine days (x 300). *B*. Section through entire wall of right ventricle of rat on deficient diet for twelve days (x 150).

diffusely infiltrated with leukocytes, these areas are reminiscent of the lesions encountered in human myocarditis, such as that following diphtheria. Alterations are found in both ventricles, but are usually scanty in the auricular musculature. Blood vessels are normal, as are the epicardium and endocardium; no mural thrombi have been observed. In animals living the longest an increased proliferation of connective tissue is found about the necrotic myocardial fibers, hence, scars of varying sizes appear. There is a reduction of the potassium content in the hearts of deficient rats.[55]

Some recent observations by Cannon and his co-workers[64, 71, 72] raise extremely interesting questions on the interrelations of potassium and sodium, with regard to the integrity of the myocardium. The Chicago workers found that the effects of potassium deficiency in protein-depleted rats could be profoundly affected by the administration of excess sodium

fibers. Cellular infiltration occurs together with proliferation of the sarcolemma nuclei. The administration of potassium is followed by repair without any excess connective tissue proliferation. Paralysis is not prominent in the rat but may be marked in the rabbit[87] and dog[82, 88] In this connection, it is of interest that periodic muscular weakness occurs in dogs made hypokalemic by excessive amounts of desoxycorticosterone[89]

Another tissue which is clearly the site of injury in potassium-deficient animals is the kidney. Changes have been described in rats[70, 76, 90] and mice.[81] In the former species polydypsia and polyuria have been noted.[91] After a week the kidneys at autopsy appear pale and swollen. As time goes on the organs increase in size and soon develop a finely pitted surface On microscopic examination the initial change is observed during the first week. This consists of fat accumulation in the tubular epithelial cells. It begins with the appearance of small sudanophilic, non-doubly refractile globules in the cytoplasm between the basement membrane and nucleus. As

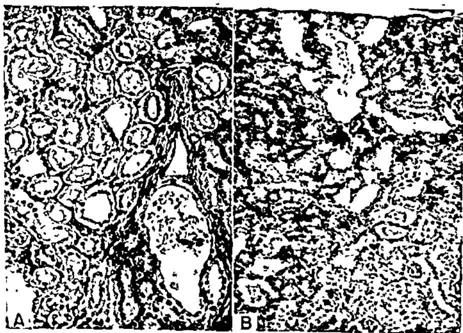


FIGURE 9. POTASSIUM DEFICIENCY.

Kidney, rat. *A* This animal had been on the potassium low ration for fifteen days Note vacuolated cells some of which contain fat A dilated tubule contains desquamated cells and debris (x 200) (Courtesy *American Journal of Pathology*). *B* This rat had been on the ration for five weeks. Note dilated tubule lined by flattened, regenerated epithelium (x 150)

ficient controls not so treated.[74] Inasmuch as sodium is known to enter the tissues to replace potassium when the organism is depleted of the latter element, investigations have been carried out to yield information on the replacement of potassium by other elements, such as rubidium, cesium, and boron[36, 75] When rubidium is substituted for potassium in a potassium-deficient diet, myocardial lesions do not appear, although the animals die after a short while. Cesium protects the heart of some animals but not all. Although low potassium diets supplemented with boron as boric acid or borax are said to permit longer survival of animals than those without added boron[35] this has not been confirmed.[36]

Since identical myocardial necroses have been observed in thiamine-deficient animals (page 200), the effect of an acute deficiency of both potassium and thiamine has been studied.[77] Lesions fail to appear even though the animals may live as long as thirty-one days. The reason for this protective effect is not clear and requires further investigation.

Another observation relates potassium to the integrity of the myocardium; cardiac lesions may be produced by administration of desoxycorticosterone (DCA). The pathogenesis of such alterations is explained by the well-known action of this adrenal hormone: to promote the excretion of potassium and to facilitate the retention of sodium. Lesions like those produced by potassium deficiency have been observed in rats treated with DCA.[78] As might be expected, when potassium-deficient animals are injected with DCA, lesions appear sooner and are more extensive than when one or the other procedure is employed alone.[79] The heart muscle of such animals treated with cortical hormone has a lower potassium content than normal.[79] It has been claimed that the hearts of animals treated with DCA develop lesions similar to those of rheumatic fever.[80] Nothing resembling an Aschoff body has ever been encountered in our own potassium-deficient material, nor do Selye's photomicrographs substantiate such a contention.

Lesions resulting from potassium deficiency have been described in the myocardium of mice[81] and dogs,[82] as well as in the Purkinje network of the hearts of calves.[83] Electrocardiographic changes substantiate morphological observations in the latter species[84] as well as in rats.[85]

When potassium-deficient rats are made to swim for prolonged periods of time their muscles exhibit tetanic contraction after cessation of the exercise.[74] This observation is of interest in relation to the tetany of potassium deficiency which has been noted in man (page 297). One might, therefore, expect lesions to occur. So they do, but not with the regularity of the changes already described in cardiac muscle. In rats,[86] rabbits,[87] and in dogs[82] well-marked lesions have been described. Such changes consist of a loss of striations followed by hyaline swelling of the muscle

fect the heart, produce renal changes, lesions similar to those in rats are made more severe as the potassium content of the diet is reduced.[93]

Studies of adrenal glands from potassium-deficient rats[94, 95] have revealed a definite effect on the cells of the zona glomerulosa. The cells become smaller; their lipid droplets increase in size but decrease in number. If DCA is administered lipid disappears completely. Such studies would appear to relate potassium, and sodium too, to the zona glomerulosa cells since potassium-sodium ratios are of importance in eliciting the changes.

No other specific alterations have been ascribed to potassium, save changes in the epiphyseal cartilage of growing animals[86] Since this tissue is so sensitive to alterations in nutrition one must hesitate to accept the lesion as one specific for potassium deficiency.

The clinical recognition of the "hypokalemic syndrome" (page 289) in *man* has prompted the study of experimental potassium deficiency in the human in order to evaluate some of the complicating factors which are seen in naturally occurring hypokalemic states An important point to realize, as Moore has emphasized, is that "hypokalemia" does not necessarily mean "tissue potassium depletion"[96]

The effects of acute potassium deprivation for periods of six and seven days were reported by Black and Milne[97] The two subjects lost 268 and 289 mEq of potassium, respectively Serum levels fell to 3.1 and 2 6 mEq liter. The serum bicarbonate concentrations rose Calcium and magnesium balances were unchanged; phosphorus balance became negative This study, as Moore points out,[96] is complicated by an excessively high sodium intake.

A more chronic study was reported by Blahd and Bassett[98] Here the subject lost 278 mEq of potassium, the serum level fell from 4 0 to 3 5 mEq per liter Pearson and Eitel[99] studied a patient who was placed on a potassium-low diet for thirty days Further drain on potassium was produced by the use of a sodium charged resin. Progressive hypokalemia without alkalosis was observed.

Moore *et al.*[96] have summarized the somewhat conflicting data on experimental and naturally occurring potassium deficiency in man and have presented their own experimental findings They call attention to the importance of (1) excess sodium intake in the face of potassium depletion, (2) extrarenal salt loss, and (3) the presence of "stress" factors. When [illegible]

renal salt loss (vomiting, diarrhea), alkalosis becomes evident, hypokalemia may then be prominent Under "stress" urinary excretion of sodium is



FIGURE 10. POTASSIUM DEFICIENCY.

Kidney, rat. This animal had been on the low potassium ration for eighty-four days. Note dilated tubules, particularly of the cortico-medullary junction (x 16).

this deposition of fat continues the epithelial cells soon become necrotic. Cellular debris and fat globules are then found in the lumens of the tubules. As early as the fourteenth day of the deficiency the tubules are found lined by flattened regenerating epithelium. As time goes on there are many tubules lined by this type of epithelium, which may pile up, and in some tubules the epithelium becomes calcified. Calcareous casts are found in the lumens of the tubules. No glomerular lesions have been observed, nor are there any changes in the renal blood vessels Studies of the activities of two enzymes, glutaminase and carbonic anhydrase have shown increased concentration as a result of potassium deficiency. Such increases appear to parallel the alterations in urinary acidity and ammonia excretion.[92]

Inclusion of rubidium in a potassium-deficient diet seemed to protect the kidneys of all the rats so studied by the present writer; cesium protected to a lesser extent.[75] Excessive amounts of desoxycorticosterone, which af-



FIGURE 11. SODIUM DEFICIENCY

Eye. Section through anterior segment of eye of rat which had been on a sodium-deficient diet for eighty-four days In the upper lid the tarsal glands are greatly dilated Note also dilated acini of lacrimal gland H and E (x 30)

during the first few weeks is normal, growth then becomes retarded and, in general, after eight to ten weeks, the animals either fail to gain at all or begin to lose weight All are dead by the eighteenth to the twenty-first week Grossly, characteristic changes appear in the eyes between the eighth and tenth week, the corneae become cloudy, the lids appear swollen and lose their hair

The tissues of such deficient rats have been studied by the present writer in association with Orent-Keiles and McCollum.[105] Microscopically, aside from non-specific athreptic changes in the reproductive systems, thymus, and bone, the only lesions to be found are those in the ocular apparatus. The pathogenesis of these changes has been interpreted as follows The progressive dilatation of the ducts of the tarsal or meibomian glands which is first seen is apparently due to obstruction of their openings by granular, pink-staining material which is found attached to the lid margins. Dilatation of the ducts accounts for the swelling of the lids which is noted during life As the dilatation becomes extreme, atrophy of the glandular epithelial cells follows Associated with these changes an alteration in the character of the epithelium of the inner lining of the lids is found Normal columnar and goblet cells are replaced by a stratified squamous epithelium. In the cornea the initial change appears to be a migration of leukocytes into the substantia propria; these are followed by an ingrowth of capillaries In the early stages of cellular and vascular infiltration the corneal epithelium shows no change, later, however, it becomes keratinized

blocked, hence alkalosis is more severe and hypokalemia becomes a serious problem.

How may the dynamic state of potassium metabolism be evaluated in the experimental animal or in man? A number of procedures may be cited:[5] (1) *Serum level.* This is valuable but may be inaccurate since hypokalemia is not necessarily synonymous with decreased intracellular potassium.[96] (2) *Tissue Analysis.* Muscle biopsies are helpful. So, too, analysis of tissue at autopsy has provided valuable information. (3) *Balance Data.* When a suitable reference substance such as chloride is used, change in outgo and intake are valuable though laborious. (4) *Isotope Dilution.* The total exchangeable potassium can be evaluated by this technique. (5) *Potassium Loading.* This procedure has been used to assess cellular stores; it also is a measure of renal function (6) *Electrocardiogram.* This is a useful adjunct particularly in clinical medicine.

SODIUM

The effects of sodium on the contractility of the frog heart was first shown by Sydney Ringer.[50] A retardation in growth of rats when such animals were placed on a synthetic diet of low sodium content was first reported by St. John[100] in 1928, the subject was studied a few years later by Orent-Keiles, Robinson, and McCollum.[101] The distribution of sodium in the organism is shown in Table 1 on page 6 The largest amounts are found in skin and bone. In both areas the cation is extracellular, just as it is blood plasma where it accounts for the major part of the basic ions.[261] Sodium may replace a part of any potassium which may be lost from cells[59]

Sodium is found in pancreatic juice, bile and intestinal secretions. Its excretion is regulated by the kidneys under the control of the adrenal cortex. Here the active principle, aldosterone, appears to play the key role.[102]

When acute sodium depletion is studied, for instance in the dog, by the removal of 23 per cent of the total body sodium by *vivodialysis*, the participation of various areas in effecting this loss is as follows: extracellular phase, 70 per cent; bone, 25 per cent; and body cells, 5 per cent.[103] The rate of removal is, with the excretion of bone, related to vascularity and water content The removal of such a large quantity of body sodium results, as might be expected, in severe, uncompensated acidosis. The sodium content of the skeleton will be commented upon elsewhere (page 146).

Orent-Keiles and McCollum[104] devised a ration which contained only 0 002 per cent sodium. When rats are placed on such a diet, gain in weight

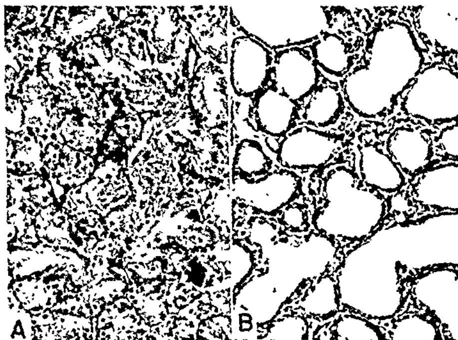


FIGURE 13. SODIUM DEFICIENCY

Lacrimal gland. *A*. Normal *B*. Section from gland shown in Figure 11. Note dilatation with flattening of epithelial cells as a result of duct obstruction. H and E. (x 200)

ectomy, cation exchange resin feeding, pyloric obstruction, and parenteral fluids and peritoneal lavage with glucose solutions. Such methods may be applied in acute experiments As yet less severe and more chronic sodium deprivation has not been studied by their use

Experimental sodium chloride deficiency in man has been studied by McCance[1473] who, in himself and a group of fellow subjects, has described anorexia, nausea, fatigue, a sense of exhaustion, and muscle cramps. As would be expected there was hemoconcentration. All such manifestations of the deficiency disappear following the ingestion of salt and water.

MAGNESIUM

The spectacular syndrome of magnesium deficiency in the rat was reported by McCollum and his co-workers in 1931.[108] Subsequent studies by the Johns Hopkins investigators and others have aided in clarifying some of the changes which take place in the animal organism when dietary magnesium is restricted

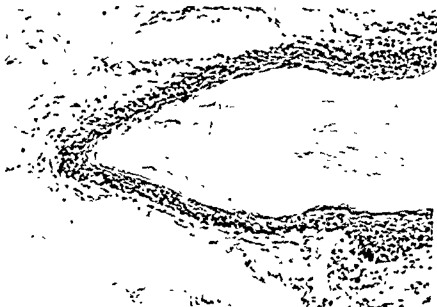


FIGURE 12. SODIUM DEFICIENCY.

Eye. Section at junction of cornea and sclera to show keratinizing epithelial lining cells, some of which are desquamating. H. and E. (x 200).

The cause or causes of the corneal changes are not clear, but are interesting to speculate upon. It is well known that the corneal epithelium is unique in that it is in contact with an hypertonic environment produced by evaporation of the tears. In the sodium-depleted animal, the sodium content of the tears is doubtless reduced with perhaps a change to iso- or even hypotonicity. The corneal epithelium conditioned normally to an hypertonic medium may be extremely sensitive to one which approaches that of normal cells. How much of a role the absence of the secretions of the meibomian glands plays is unknown.

Low sodium diets may lead to changes in the adrenal glands.[94, 95] Increase in the size of glomerular zone and its cells has been described. Such studies have not been well-controlled, however.

The effects of sodium deprivation have been studied in dogs in which observations were made over a period of eight weeks. Loss of weight, dryness of the skin, and loss of hair are described, the eyes show no changes.[106]

Sodium depletion can, as just noted, be produced by dietary restriction. One may make the deficient state develop more rapidly and become more severe by certain adjuvant procedures.[107] These include· peritoneal dialysis with glucose, thermal sweating, excess water intake in a hot environment, parenteral glucose administration, mercurial diuretics, adrenal-

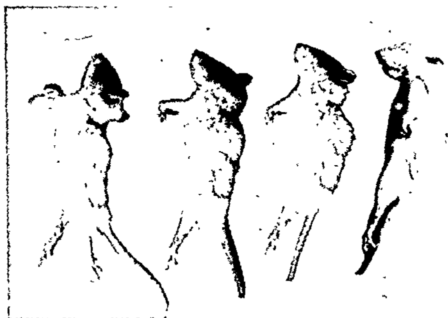


FIGURE 14 MAGNESIUM DEFICIENCY

Tetany From right to left are shown several stages in a convulsive seizure resulting from magnesium deficiency In the first stage there is great spasticity with hyperextension This is followed by relaxation which may be in turn followed by rigidity and opisthotonos The animal *may recover or die* (Courtesy of Dr Maurice Sullivan and *Archives of Dermatology and Syphilology*)

thorax, fore paws clenched and hind extremities extended This spastic condition may give way to clonic contractions in which the fore limbs are alternately drawn up to the chest and extended from the body Next the animal suddenly leaps into the air at the same time spinning laterally several times, or it may "curl up" with marked flexion of all extremities, or it may do neither This is marked cyanosis Associated with the convulsive seizure is regurgitation of the stomach contents into the esophagus and mouth, as sacrifice experiments during this period have shown.

"Within a short time the animal rears from the dorsal or lateral recumbent position in an attempt to stand, but its extremities will not support it The animal buries its head in its outstretched fore limbs and propels itself forward entirely by its hind limbs which, however, are so extended with paws hyperextended that the dorsal, not the plantar surface, bears the weight. Instead of forward motion, fine tremors may appear over the body. Throughout this stage the eyeballs are retracted

Magnesium is widely distributed in the tissues; here its intracellular concentration is second only to that of potassium. The greatest amount is found in the bones from which it would appear to be readily mobilized This cation accounts for 0.5 to 0.7 per cent of bone ash.[109] Magnesium is also present in plasma, though it furnishes only a very small proportion of the basic ions.[261] The irritability of muscle and nerve is affected by changes in concentrations of magnesium; an excess produces narcosis,[110] while a decrease leads to hyperirritability.[111] A relationship of magnesium to protein metabolism is evinced by an increased need for this cation when protein intake is elevated [112]

Magnesium ions, like those of many other elements, are necessary for the activity of certain enzymes.[27] Here they participate either as activators or as specific components of the enzyme. In the first category, magnesium is extremely important, for there are a number of sugar phosphorylating enzymes with which it appears to play a dominant part in the formation of an enzyme–substrate complex. Magnesium would appear to play the most important role in the glycolytic cycle just as manganese does in the Krebs cycle.[113] With respect to the second category of effects, magnesium appears to be an integral part of the enzyme, inorganic pyrophosphatase.

The effects of magnesium deficiency have been studied in rats, rabbits, dogs and calves. In the initial observations of McCollum *et al.*[114] rats were placed on a diet containing only .2 mg. per cent magnesium. A specific syndrome developed which was characterized by dilatation of the cutaneous vessels, hyperirritability and convulsive seizures. The latter could be precipitated by external stimuli of various types. The first attack proved fatal in about 80 per cent of the animals. The following description graphically portrays the course of one of the seizures: "The excitable animal startled by sound, races at rapid speed in a wide circle until it finally falls on its side. The entire body of the animal is now rigid, with head stretched back, forelimbs extended at three upper joints and flexed at the metacarpophalangeal joint, and hind limbs extended backward. So fixed are the jaws that often the tongue is perforated by the clenched teeth. The skin presents a waxy appearance. All respiratory movements cease during the attack and return with the relaxation of the musculature. Priapism may appear at this time and persist until death.

"The stage of spasticity is succeeded by a period of relaxation lasting only a very short time. While still lying on its side the animal exhibits twitching in various regions, or paddles rapidly with all extremities. Coincident with this behavior, the animal's eyeballs become more prominent, the ears stiffen and project backwards against the side of the head and the fur stands erect. Then reappears a tonic spasm in which the rigid body assumes a typical position with the fore limbs pressed tightly against the



FIGURE 15 MAGNESIUM DEFICIENCY

Skin *A* Extensive edema of the digits and the plantae of the paws of a rat Note ulceration of the surface of the latter *B* Skin from tail to show hyperkeratosis, acanthosis, vacuolization of some of the epithelial cells, and diffuse cellular infiltration in the corium. (Courtesy of Dr Maurice Sullivan and *Archives of Dermatology and Syphilology*).

"Following the convulsive stage comes the recovery stage, doubtless dependent on exhaustion. During this period there is moderate cyanosis of skin, coldness of the extremities, lacarimations from the dull, shrunken eyes, champing of the jaws and drooling from the mouth. A hemorrhage may issue from the nose and orbit and bloody frothy fluid consisting largely of regurgitated stomach contents mixed with blood may bubble from the mouth. No urinary or fecal incontinence is seen during the attack."

Similar convulsions have been noted in rabbits,[115] dogs[116] and calves.[117] Careful microscopic examinations of the nervous tissues of magnesium-deficient animals have not been reported. Studies of the action of certain drugs on magnesium-deficient rats led Tufts and Greenberg[118] to conclude that the locus of action of the sensory stimuli, which produces the seizures, is in the midbrain. These investigators feel that tetany syndromes resulting from either calcium or magnesium deficiencies differ since the muscle spasms in the animals depleted in the former cation are abolished by curare, while in those deprived of magnesium, they are not. In view of our inadequate understanding of the true nature of tetany it would seem unwise to argue that magnesium deficiency can or cannot lead to this syndrome. It is of interest, however, that the electrical threshold is reduced in magnesium-deficient rats, as it is in tetany resulting from other causes.

Chemical studies on magnesium-deficient rats have shown an early and abrupt fall in serum magnesium from a normal of 2.96 mg. per cent to 0.81 mg. per cent.[119] After this initial fall the serum magnesium slowly rises. As would be expected, the urinary excretion of the ion is greatly diminished. There is a concomitant retention of calcium.[120] Certain other abnormalities in blood chemistry have been interpreted to result from a general nutritive failure, since such changes occur later in the deficiency. For instance, increased cholesterol and decreased fatty acid values,[119] together with a reduction in serum alkaline phosphatase activity,[121] have been observed. Similar reductions in the magnesium content of whole blood and serum have been noted in rabbits.[115]

McCollum *et al.*[114] have called attention to the appearance of tachycardia as acute magnesium deficiency develops; electrocardiographic studies in rats reveal sinoauricular block.[118]

Microscopic examination of the tissues of magnesium depleted rats has revealed widespread changes[122] which have been characterized as an inflammatory reaction in loose mesenchymal tissues about blood vessels of precapillary and capillary size. The lesions progress through an acute stage with granulocytes, through a necrotic stage with macrophages, ending with healing characterized by connective tissue proliferation. Thus it would appear that blood vessels are primarily affected. The changes found may represent the anatomic basis for the increased capillary permeability which has been postulated.

by competent oral histologists,[130, 132, 133, 134 135, 136] the following changes seem well-established An early manifestation is retardation of dentine formation, particularly that of the labial surface, which comes to be half or less the width of the lingual dentine. Peculiar striations in the dentine appear; these may be due to variations of growth similar to those which are seen in bones.The odontoblasts are responsible for the changes in the dentine since these cells become atrophic and soon are inclosed on all sides by dentine. In a similar fashion the ameloblasts become atrophic, as a result enamel formation is retarded and the resultant covering is hypoplastic Calcified stones are found in the pulp of magnesium-depleted teeth

Chemical analyses have shown no great decrease in the absolute magnesium content of the rat's incisor.[137] This is unlike the situation in bone where magnesium has been shown to decrease and calcium to increase in the early stages of the deficient state.[138, 139] This is, of course, to be expected in view of our present understanding of the dynamic equilibrium of various inorganic ions with the bone crystal (page 146) It is of interest to note that one of the studies of the magnesium content of bone, which showed the extreme lability of the element, antedated the classical studies of Hevesy[131] with radioactive phosphorus

In addition to the rat, rabbit, and dog, extensive studies have been carried out on calves which were placed on low magnesium rations consisting of a synthetic milk containing .35 to 50 mg magnesium per 100 ml[117, 140, 141 142] Evidences of deficiency were opisthotonus, hyperirritability, tremors, and finally convulsions. Serum magnesium levels fell, calcium and phosphorus concentrations remained normal Bone magnesium was decreased by 30 per cent. Despite these decreases in serum and bone magnesium the soft tissue content of this ion was not reduced Hence, it is unlikely that any of the physiological or anatomical changes which are encountered in the deficient animal can be ascribed to the effect of magnesium deprivation on enzyme systems which this cation is known to affect. Analysis of bone biopsies have proved to be an important tool for studying the development of the deficiency syndrome in calves

In man, experimental magnesium deficiency has been studied[1433] No symptoms, save weakness, were noted. Evidence for depletion of magnesium was demonstrated chemically

CHLORINE

The indispensability of chlorine in the diet was first shown by Orent-Keiles, Robinson and McCollum in 1937, when they placed rats on a synthetic, low-chloride diet, the animals failed to grow in normal fashion.[101]

Dilatation of the cutaneous vessels is one of the prominent features of magnesium deficiency which McCollum and Orent[108] first described; such hyperemia lasts about a week. In those animals which survive the ensuing convulsions, edema of the paws and ears as well as changes in the skin may be noted. Careful studies of the pathogenesis of the cutaneous lesions have been reported by Sullivan and Evans.[123] From the fourth to the eighth day of deficiency, erythema and edema become prominent in the ears and paws over the trunk. Microscopically, the vessels of the cutis are dilated; fluid and cellular infiltration are observed in the corium. At this stage no alteration in the epidermis nor any loss of hair is found. Later, however, a loosely laminated hyperkeratosis appears and is followed by a patchy distributed acanthosis. Individual cells become vacuolated and display pycnotic nuclei. No changes can be detected in the sebaceous and coil glands. Sullivan and Evans[123] were unable to substantiate a claim[118] that signs of magnesium deficiency are affected by the vitamin B content of the diet, nor could the observations of MacCardle *et al.* be confirmed. By spectrographic[124] and micro-incineration[125] studies, the latter investigators found a decrease in the magnesium content of the skin in cases of human neurodermatitis. From this they postulated that neurodermatitis and magnesium deficiency are identical or similar diseases. Sullivan and Evans[126] have conclusively shown that the pathologic manifestations of these two syndromes are quite different.

The renal lesions which may appear in magnesium-deficient rats have been inadequately studied. The picture is further complicated by the use of diets which may not have furnished all necessary nutrients. The following changes have been noted: extreme degeneration of tubular and glomerular epithelium with calcareous deposits in the lumens of the tubules;[127] calcium deposits in the straight and collecting tubules with cystic dilatation of the structures above;[128] "extreme degeneration of the tubules and glomeruli and deposits of calcareous material in areas of degeneration."[123] Increased urinary volume and proteinuria, but no hematuria or casts have been observed. The hypoproteinemia which ensues, may be followed by edema.[129] More complete studies of the renal changes are much to be desired.

Alterations which have been described in the liver are even more fragmentary and difficult to evaluate; "hyperemia, perivascular edema, and occasional disintegration of liver cells" have been recorded.[123]

Studies of magnesium deficiency by the Johns Hopkins investigators revealed changes in another area, the teeth. Here, extreme hypertrophy of the gums, the result of subepithelial connective tissue proliferation, was observed.[130] Striations were noted in the dentine as well as alterations in the ameloblastic layer. The dental structures have been carefully studied

on the experimental regimen for as long as ninety days. Histological studies of such animals were not performed These observations have been confirmed by other investigators utilizing diets of various chloride concentrations.[143, 144] When a ration containing only 20 mg. per cent chloride was fed to rats a retardation in growth was found, together with a reduction in the sodium, potassium, and chloride content of the tissues and an increase in calcium and phosphate concentrations. When a similar diet containing only 12 mg. per cent chloride was employed,[145] rats dramatically exhibited a conservation of chloride; for after the animals have been on a deficient diet for only a few hours the urinary chloride excretion decreased to virtually zero Compared with controls which excreted 110 to 170 milligrams of chloride per day, the deficient animals excreted only 0 5 to 1 2 mg per day. Such rats also showed a fall of serum chloride from 295 mg. per cent to 252 mg. per cent An increase in the CO_2 combining power from 58.8 to 72 3 volumes per cent was also found. Manifestations of tetany have not been observed. It would be interesting to augment the chloride deficiency produced by dietary means with that produced by removal of gastric secretions.

An even lower chloride-deficient diet (2-5 mg per cent) leads to a reduction of the chloride concentration in skin, muscle, liver, kidney, testis, brain, stomach, lungs, and total carcass.[146] The amount of chloride in the heart and spleen is increased An interesting change has been described in the kidney [illegible] institution of [illegible] in the kidney [illegible] and possible elevation of phosphate concentrations in the convoluted and collecting tubules, a precipitation of calcium salts occurs in these structures. This leads to obstruction of the lumens and initiates a reaction in the tubules and peritubular tissues Since most of the tubules may become obstructed, one finds a kidney which resembles "a shell filled with fluid, consisting of a much thinned cortex with pelvic epithelium forming folds separated in part from the compressed cortical zone " No lesions are found in the other tissues of these animals save an arrest of spermatogenesis which is doubtless the result of inanition

CALCIUM

Calcium is one of the major components of bone where 99 6 per cent of the total content of this cation is found (page 146). Its role in certain physiological processes is clear enough. Hence, decreased intake in the diet, excess loss via the intestine or kidneys, or poor regulation of serum

Chloride ions occur principally in the extracellular fluids, accounting for about two-thirds of the anionic constituents.[261] Chloride, of course, is an important secretory product of the gastric mucosa. Strangely enough, little else is known of the function of chlorine in the animal organism except for its activation of the salivary enzyme, ptyalin.

A histochemical method has been used to indicate the distribution of chloride in striated muscle.[56] Here it is found only in the intercellular spaces; none is present in the muscle fibers themselves.

Physiological as well as morphological observations have been made by several groups of investigators on chloride-deficient animals. The morphological observations are somewhat inadequate, however. Using a diet of unknown, though low, chloride content, Orent-Keiles *et al.*[101] could detect no changes other than a disturbance in growth even after rats had been

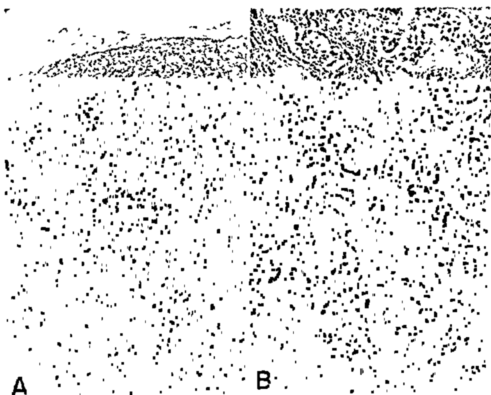


FIGURE 16. CHLORIDE DEFICIENCY.

Kidney. Tissue from rat which had been on a chloride deficient diet (2-5 mgm. per cent) for fifty-six days. *A.* Low power (x 35) to show smooth surface. Small foci of damaged tubules can be made out. *B.* Higher power (x 150) to show atrophic, collapsed tubues with granular material in their lumens. H. and E (*Courtesy of Drs* E. Lowenhaupt and D. M. Greenberg).



FIGURE 17 CALCIUM DEFICIENCY

there are broad bands of osteoid about the few spicules of calcified cartilaginous matrix This area shows great disorganization H and E, silver nitrate (x 50)

that time, an unequivocal, uncomplicated calcium deficiency syndrome could not be produced Be that as it may, these now classic experiments clearly elucidated the role of vitamin D in nutrition.

The first demonstration of uncomplicated calcium deficiency was reported in 1937 when Martin[154] prepared a diet adequate in all respects save that it contained only 3 mg of calcium per 100 grams. The character-

levels as a result of parathyroid gland malfunction or absence may lead to certain now well-recognized effects.

One of the earliest studies which demonstrated the importance of calcium was Ringer's report on the need for this cation, in association with those of sodium and potassium, in maintaining the integrity of the contractile mechanism of the heart.[50] Soon after this its role in the coagulation of blood was demonstrated.[148] The precise action of calcium ions in this complex phenomenon has yet to be completely elucidated; calcium is needed for several of the many reactions which are necessary to transform fibrinogen into stable fibrin (page 466). The effect of calcium ions on the irritability of nerve and muscle and its relation to tetany should be recalled[149] (see page 295). There is some experimental evidence that calcium controls the permeability of capillaries by virtue of its enhancement of the solubility of "intercellular cement substance," which may be a calcium-proteinate.[150]

The relation of calcium to tetany was demonstrated by MacCallum and Voegtlin[151] who first showed the reduction in serum calcium which follows removal of the parathyroid glands. Today it is clear that the hormone or hormones liberated by these structures help to govern calcium homeostasis Excess parathyroid activity causes hypercalcemia and hypophosphatemia with hypercalcuria and hyperphosphaturia. On the other hand, decreased activity or removal of the parathyroids leads to hypocalcemia and hyperphosphatemia with hypocalcuria and hypophosphaturia[152]

The intestinal absorption of calcium is regulated, or rather enhanced, by the vitamins D[585] Further discussion of the metabolism of calcium will be found on page 148

Calcium deficiency has been produced experimentally by dietary means in rats, rabbits, guinea pigs, and dogs. Naturally occurring calcium deprivation is discussed elsewhere (page 361). Aside from certain expected chemical alterations which will be summarized below, calcium deficiency leads to widespread hemorrhages, lesions in the gastrointestinal tract, cataracts, parathyroid enlargement, and, of course, rickets, which is discussed more fully on page 149.

Prior to 1937, many reports had been recorded on animals to which low calcium rations had been fed. For instance, in a study reported by Voit young dogs were given a diet of horse meat.[153] Such investigations antedated Pommer's description of rickets in 1885.[527] The rations employed by Voit and those of others were deficient not only in calcium but in other essential nutrients. When one turns to the more modern studies of McCollum, Park *et al*[530, 571] who employed cereal grains as the main constituents of the diets with which to produce low-calcium rickets, the same criticism is still valid. For, since synthetic diets could not be employed at

by the twenty-third week. At autopsy, widespread hemorrhages are found in the tissues, extravasation of blood is prominent in the nervous system, especially of those animals which exhibit paralysis before death. Hemorrhage and paralysis are common in the young born of calcium-deficient females.[157] Bleeding is also a prominent feature of a calcium-deficient syndrome described in dogs.[154] It is unfortunate that microscopic studies have not been reported in these two species, since it would be of interest to determine, if possible, whether actual damage to capillary endothelium can be demonstrated or whether the hemorrhages are incident to ordinary "normal" trauma to vessels. That the endothelium may be damaged is suggested by the work of Chambers who has presented evidence that "intercellular cement substance" may be a calcium-protein complex.[150] If this be true, one would suppose that actual defects are present in the capillaries of calcium-deficient animals. Presently, the pathogenesis of the hemorrhages in calcium deficiency is just as mysterious as it is in vitamin K deficiency, where a similar question remains to be settled (page 172).



FIGURE 19 CALCIUM DEFICIENCY



FIGURE 18. CALCIUM DEFICIENCY

Upper epiphysis of rat's tibia *A* Cartilage shaft junction of section shown in Figure 17 Note disorganization of cartilage cells, many of which appear to be empty. There is compression of the connective tissue beneath, with broad bands of osteoid about the spicules of calcified cartilage matrix. H and E, silver nitrate (x75) *B* Microradiograph of undecalcified section of same block shown in *A* Note outlines of cartilage cells and dense calcified material (bright zone) which blends into the bands of osteoid (x 75).

istic syndrome which developed in dogs fed this ration consisted of widespread hemorrhage, prolongation of the coagulation time, inflammation of the gastrointestinal tract, and "osteoporosis."

A most severe calcium deficiency has been reported in young rats by Boelter and Greenberg [155, 156] When such animals are placed on a diet containing only 0 01 per cent calcium, growth is retarded in from four to five weeks; after seven to ten weeks, the animals exhibit a generalized decreased sensitivity and reactivity Coincident with this the serum calcium falls to about 5 mg per cent; tetany, however, does not appear. Paralysis of the hind legs may be noted and, when the deficient animals are stimulated by galvanic shocks, collapse occurs Sixty per cent of the rats succumb

by the twenty-third week. At autopsy, widespread hemorrhages are found in the tissues; extravasation of blood is prominent in the nervous system, especially of those animals which exhibit paralysis before death. Hemorrhage and paralysis are common in the young born of calcium-deficient females [157] Bleeding is also a prominent feature of a calcium-deficient syndrome described in dogs.[154] It is unfortunate that microscopic studies have not been reported in these two species, since it would be of interest to determine, if possible, whether actual damage to capillary endothelium can be demonstrated or whether the hemorrhages are incident to ordinary "normal" trauma to vessels That the endothelium may be damaged is suggested by the work of Chambers who has presented evidence that "intercellular cement substance" *may be a calcium-protein complex.*[150] *If this be true,* one would suppose that actual defects are present in the capillaries of calcium-deficient animals. Presently, the pathogenesis of the hemorrhages in calcium deficiency is just as mysterious as it is in vitamin K deficiency, where a similar question remains to be settled (page 172).



FIGURE 19. CALCIUM DEFICIENCY.

Cortex of diaphysis of rat *A* Section through cortex of shaft of bone shown in Figures 17 and 18. *Note bone (black) surrounded by osteoid along periosteal and endosteal* surface. H. and E , silver nitrate (x 75). *B* Microradiograph of serial section of same block to show calcified bone surrounded by less dense zone of osteoid The deposition of inorganic material is irregular in the matrix (x 75).

Boelter and Greenberg[155] have not found any abnormalities in the gastrointestinal tract of the rat. However, other observers are not in agreement, since lesions have been described in the antrum of the stomach, though not in the fundus or rumen.[158] Such changes consist of hyperplasia of the lining epithelium with necrosis and hemorrhage. It is of interest that in dogs hemorrhagic and ulcerated gastric and intestinal lesions have been described.[154]

Reproduction of rats on a low-calcium diet has also been studied.[157] Fertility rapidly decreases and the animals soon fail to mate. In addition, those females which give birth to young have insufficient milk to nourish their offspring. Whether these abnormalities are the result of inanition or of calcium deficiency are questions which remain to be investigated further.

When moderate amounts of calcium salts are injected intravenously into normal rats they are perfectly innocuous; administration of similar quantities to calcium-deficient animals results in rupture of the right ventricle of the heart,[155] an extraordinary phenomenon.

The cause of the neurological disturbances which are noted in calcium-deficient animals is not at all clear. Tetany does not appear but paralysis, particularly of the hind legs, has been noted in rats[155] and dogs.[154] In addition, tonic or clonic convulsions are said to occur in the latter but not in the former species. Intracerebral hemorrhage has been noted in the rat but more studies, both physiological and anatomical, of the nervous tissues of calcium-deficient animals are needed.

Several other miscellaneous effects of calcium deficiency have been described and should be studied further. In the rat, unpurified, low-calcium diets lead to an increase in size of the parathyroid glands; the change is said to be due to both hyperplasia and hypertrophy of the cells; an increase in the number of osmophilic cells and a change in the complexity of the Golgi apparatus have also been noted.[159] A quantitative study of the relationship of calcium intake to parathyroid volume has been reported.[160] Here the calcium and phosphorus content and ratios in a synthetic diet were varied. A close inverse proportionate was found between the dietary calcium-phosphorus ratio and the volume of the parathyroid glands of adult rats. Between the serum levels of 7.3 and 11.9 mg. per cent the parathyroid volume varied in a linear fashion. The serum inorganic phosphate level was less related to parathyroid size.

When rabbits are placed on a low-calcium diet, lens opacities may be noted.[161] Such changes in the lens are observed during the second week of the deficiency and consist opthalmoscopically as slits, vacuoles and dots near the equator of the lens. The opacities then progress out toward the anterior and posterior suture lines. Calcium deficiency in such animals has

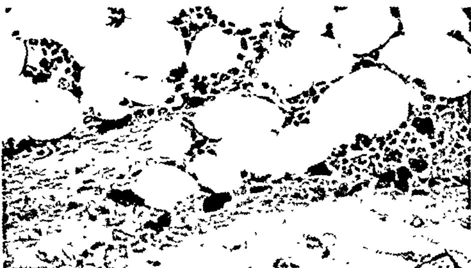


FIGURE 20. CALCIUM DEFICIENCY

Mast cells Marrow elements from tibia of rat which had been placed on a calcium-deficient synthetic diet for five weeks Large numbers of mast cells are present (×200)

been corroborated by the appearance of tetany and a reduction in serum calcium concentrations

A most interesting finding, which was reported by the Johns Hopkins group in the bones of rats which had been fed a low-calcium, cereal grain ration,[571] was the presence of large numbers of cells containing basophilic granules The appearance of the bones was described as follows. "Bones which showed evidences of resorption invariably contained numbers of large cells, the cytoplasm of which was filled with large basophil granules They were found in the region of the bone which was being hollowed out to form a marrow cavity, but were not seen in the medulla itself. They were also present in large numbers in the interstices of the bony lattice work which represented the cortex They were not present in the metaphysis to any extent, and were not found in the centers of ossification."

The presence of large numbers of what are obviously mast cells has recently been rediscovered by Urist and McLean[1474] and ourselves.[1251] The diet used by McCollum *et al*[571] was made up of cereal grains, sodium chloride and butter fat That employed by Urist and McLean[1474] contained 79 per cent corn, 20 per cent gluten and 1 per cent NaCl. Our own experiments, which were not designed to study rickets, were first carried out with diets of corn meal alone. When mast cells were found, our immediate reaction was that they might not be related to calcium deficiency at all, since corn is lacking in so many other essential nutrients. So, too, the pres-

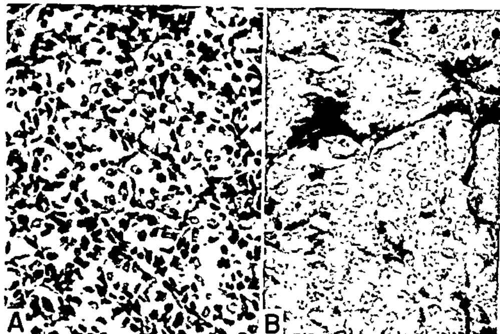


FIGURE 21. CALCIUM DEFICIENCY.

Parathyroid gland *A* Parathyroid from normal rat. *B* Parathyroid from calcium-deficient rat. Note difference in number and size of nuclei and abundance of cytoplasm in comparison with *A* H. and E. (x 200).

ence of goiter and other alterations made us consider other factors. We have since carried out a number of observations not yet published which indicate that iodine, amino acid and vitamin deficiencies do not lead to accumulation of the mast cells. On a synthetic calcium-deficient diet, such as that employed in the experiments of Boelter and Greenberg,[155] mast cells continue to appear though not in as large numbers as on corn diets. We are currently investigating the possibility that some positive factor in corn may be implicated We have never encountered mast cells in human rachitic material. Moreover, we have restudied the bones from phosphorus-deficient animals[164] and have failed to find mast cells. Their significance in these calcium-deficient rats is, at the moment, a complete mystery.

PHOSPHORUS

Because of its widespread distribution, phosphorus has been regarded as an essential nutrient for some time. The first pointed experiments were performed in 1918 by Osborne and Mendel[52] who showed that this ele-

ment is necessary for the growth of rats. The experiments of McCollum *et al*[531] and of Sherman and Pappenheimer[532] soon demonstrated the importance of phosphorus in the production of rickets It only remained to show that phosphorus deficiency produced pathological effects when all other known nutrients, including vitamin D, were present in the diet This was accomplished by Schneider and Steenbock[182] in 1939.

Approximately three-quarters of the body's store of phosphorus is found in the skeleton. In addition to its important role in the formation of the bone salt, phosphorus is also one of the most important, perhaps *the* most important element, excluding carbon, hydrogen, oxygen, nitrogen and sulfur, in physiological processes, since it is concerned with the liberation of energy for muscular contraction, secretion by the kidney, et cetera Its functions are too familiar to require anything but brief mention: in the metabolism of carbohydrate by muscle and other cells; in lipoid metabolism (lecithin, cephalin, et cetera), in protein metabolism (nucleic acids, creatine), and



FIGURE ...ORUS DEFICIENCY.

Upper epiphysis of tibia Note increase in width of cartilage and disorganization of lower-most strata of cells Calcification of the cartilage matrix is defective. The trabeculae in the metaphysis are made up of remnants of calcified matrix surrounded by osteoid H and E, silver nitrate, undecalcified section (x 50).

as a constituent of certain enzymes (cocarboxylase, flavo-proteins, pyridine nucleotides).

Because of the interrelationships of phosphorus, calcium, vitamin D and the parathyroid hormone, further discussion of phosphorus metabolism will be found in the section on vitamin D, page 141.

Day and McCollum[163] *have reported the preparation of a diet with* which to study the effects of phosphorus deficiency; their ration, which contains only 0.017 per cent phosphorus, has an adequate calcium (0.4 per cent) and vitamin D (380 I.U per cent) content. When young rats are placed on this diet, an extreme retardation in growth is found; the animals soon appear unkept and become very inactive. When the tissues of such animals are examined,[164] aside from the manifestations of profound inanition, the only *specific gross or microscopic alterations are found* in the skeletal system, where extreme rickets is present. Due to extensive changes in the ribs, the thorax is greatly deformed and consequently reduced in capacity. Since the lungs become extremely atelectatic, it is quite apparent that respiratory difficulty contributes in large measure to the fatal outcome which *occurs after eight or nine weeks on the deficient diet. The thoracic* deformities are similar to those which Park and Howland[165] described many years ago in rachitic children.

Microscopically, the alterations in the bones of these phosphorus-deficient rats are typical of rickets; changes appear after the animals have been on the experimental diet for *only one week. In the latter stages of* the deficiency, that is, after the sixth or seventh week, rickets becomes less conspicuous due to the slowing and virtual cessation of growth. In fact, towards the end, as one might expect,[164] the rickets begins to heal A description of the histological changes will not be detailed here since the *pathologic anatomy of rickets is described on page 149 Other studies of* rats[166] and dogs[167] on phosphorus-low diets have revealed little else save rickets.

Metabolic observations[163] on phosphorus-deficient rats reveal a continual negative balance of this anion. For instance, when the total intake *of phosphorus by the deficient animals* was 34 mg. for an eight week period, the total fecal and urinary outputs were 69 and 10 mg, respectively. Paired-fed controls ingested 592 mg., of which 83 and 124 mg. were excreted in feces and urine, respectively. The metabolism of calcium in these rats is also of interest. Total calcium intake of the phosphorus-deficient *animals for the eight week period* was 853 mg.; controls ingested 822 mg. Fecal and urinary excretions were 225 and 887 mg. for the deficient and 125 and 192 for the controls, respectively. Thus, a profound loss of calcium occurred as a result of the phosphorus deficiency. *This deficit was precipitous* during the first two weeks; almost two-fifths of the entire deficit

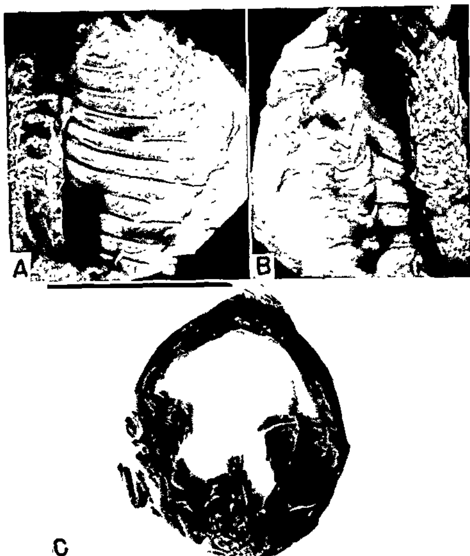


FIGURE 23. PHOSPHORUS DEFICIENCY

Thoracic ribs and cage. *A* Ribs from rat on phosphorus-deficient diet to show multiple fractures. *B* Ribs from more chronic phosphorus deficiency to show severe deformity of costochondral junctions as result of respiratory movements and muscle pull. *C* Thorax of rat chronically deficient in phosphorus to show marked deformity and reduction in capacity Interference with expansion of the lungs would be expected as shown in Figure 24



FIGURE 24. PHOSPHORUS DEFICIENCY.

Lungs A Lung from normal rat. Tissue is well distended (aerated) save for pe- ... of the thorax. B Section of lung

occurred during this interval. The total loss for the deficient animals was 259 mg.; the controls, on the other hand, retained 505 mg. The loss of calcium (259 mg) by the deficient rats was much greater than that of phosphorus (45 mg) If it is assumed that most of the former came from bone, then, since the Ca: P ratio of bone is 2 0, approximately 129 mg. phosphorus should have come from the skeleton. However, the total loss was only 45 mg , hence, some 84 mg. of phosphorus must have been taken up by the soft tissues. No appreciable derangements in the metabolism of sodium, potassium or magnesium were noted in these phosphorus-deficient ani-

mals. As might be expected, nitrogen retention was *considerably less in* the depleted group

The experimental diet of Schneider and Steenbock,[162] which was referred to above, contained somewhat more phosphorus (0.4 per cent) and calcium (.57 per cent), as well as six to twelve times as much vitamin D, than the ration developed by Day and McCollum.[163] The former workers observed citrate calculi in the kidneys, ureters and bladders of their rats.[168] They concluded that the vitamin D present in the diet changed the preference of phosphorus for the soft tissues to that for bone, a hypothesis which is of great theoretical interest, since it relates to the mode of action of vitamin D. This problem has been further studied by altering the calcium content of low phosphorus diets, with and without added vitamin D.[169] The negative calcium balance of the low phosphorus diet can be changed to a positive one by increasing the calcium intake, if the diet also contains vitamin D. Such is not possible in the absence of this vitamin. When calcium is retained, growth is decreased and the calcium contents of serum and bone are increased. This decrease in growth may be interpreted as due to an inability of the tissues to obtain phosphorus since it is being deposited in bone. The above studies were performed on young animals which were growing, though at reduced rates.

Citrate calculi have been produced in adult rats which were placed on a normal calcium-low phosphorus ration.[170] In addition, the effects of protein depletion were studied in such animals. An increased concentration of serum calcium results and is accompanied by calcuria. With lower protein values non-protein bound calcium values increase. Since citrate content increases, calcium citrate calculus formation would seem inevitable.

Hypercalcemia, with serum levels as high as 20 mg. per cent, has been observed in puppies on low phosphorus diets.[167]

Parathyroid hyperplasia has been described in the phosphorus-deficient rat.[159] These observations are open to question, since purified diets were not employed. In our own experience, enlargement of the parathyroids has not been encountered when animals were placed on a diet adequate in all nutrients save phosphorus.[164]

SULFUR

Sulfur has been known from earliest times. The importance of inorganic sulfur in metabolism has been dwarfed, particularly in recent years, by *interest in the 2 sulfur-containing amino acids,* methionine, which is in the essential group, and cystine, which is non-essential.

Sulfur is found in a wide variety of compounds in the organism and is

physiologically one of the most important elements. For instance, it occurs in amino acids (methionine, cystine), in certain hormones (estrogens, insulin), in a variety of enzymes, in certain vitamins (thiamine, biotin), in complex carbohydrates (chondroitin sulfuric acid) and in lipids (sulfatides).

The functions of ingested inorganic sulfur are not at all clear. The biochemical relationships of the organic sulfur compounds, i.e., the amino acids, are discussed elsewhere (pages 96 and 251). When inorganic radioactive sulfur is fed to rats, it does not appear in the cystine of the hair or the carcass.[171] Nor can this species utilize elementary sulfur in lieu of cystine or methionine; for when sulphate is incorporated in the diet of animals deficient in these amino acids, virtually all finds its way into the urine where it is excreted in an inorganic form.[172] Finally, if rats are fed colloidal radioactive sulfur, none can be detected in their body protein [173] Such studies make it apparent that inorganic sulfur cannot be utilized to build or replace sulfur-containing amino acids. On the other hand autoradiographic studies with S^{35} clearly indicate that this material may readily be incorporated into many tissues, particularly where sulfomucopolysaccharides are known to be present in considerable quantities.[174]

A study of inorganic sulfur deficiency in rats, which was reported some years ago,[175] revealed no adverse effect on growth in animals which had been placed on a sulfur-poor diet. On the other hand, the importance of inorganic sulfur for a wool producing ruminant such as the lamb has been clearly shown.[176] When young growing animals were placed on a purified diet of low inorganic sulfur content with urea as the source of nitrogen, growth was poor and death ensued. Wool growth continued, though at a reduced rate, which would appear to indicate that it had a higher priority than general growth, or even the maintenance of other tissues. In such an experiment as this the bacterial flora obviously play a most important role. Similar syntheses of sulfur amino acids by microorganisms have been noted in goats and ewes [177]

COPPER

The indispensability of copper for the animal organism was announced in 1928 by Hart, Steenbock, Waddell, and Elvehjem.[178] These investigators showed that rats develop a severe anemia when they are placed on a milk

is richer in its content of this element than is any other tissue, except liver.

The following concentrations of copper in mg per kilo of dry weight of certain human tissues have been reported: liver, 40.2; cerebellum, 28.8, cerebrum, 18.1; kidney, 14.1, heart, 13.4, pancreas, 8.7; and muscle, 6.4

The mechanism whereby copper is absorbed from the intestinal tract is unknown Circulating copper is found bound to an alpha-globulin called ceruloplasmin Plasma copper increases under a variety of conditions, hypocupremia, in man, at least, is rare. The functions of copper have to a large extent been revealed as a result of studies on copper-deficient animals.[180] These will be detailed below

Areas in which changes have been described as a result of copper deficiency in various species include the hematopoetic tissues, bone, hair, and the nervous system

Anemia has been observed in copper-deficient rats,[181] rabbits,[182] dogs[183] and swine[184, 185] The latter species has been most extensively studied, changes found by Cartwright and his co-workers[185, 186, 187, 188] are summarized in Table III. A marked microcytic, hypochromic anemia develops Leucopenia is usually present, platelets are not much changed In the bone marrow, which is hyperplastic, the cells of the erythroid series tend to have larger nuclei and have an increased diameter.[185] More cells of the baso-

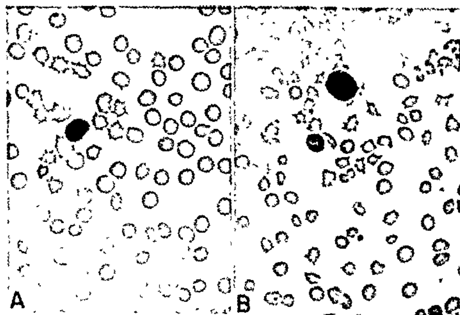


FIGURE 25 COPPER DEFICIENCY.

Peripheral blood, pig *A* Normal *B*. Cells from copper-deficient animal Note microcytosis and anisocytosis Wright's stain (x 580).

physiologically one of the most important elements. For instance, it occurs *in amino acids* (methionine, cystine), in certain hormones (estrogens, insulin), in a variety of enzymes, in certain vitamins (thiamine, biotin), in complex carbohydrates (chondroitin sulfuric acid) and in lipids (sulfatides).

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COPPER

The indispensability of copper for the animal organism was announced in 1928 by Hart, Steenbock, Waddell, and Elvehjem.[178] These investigators showed that rats develop a severe anemia when they are placed on a milk diet and that this anemia does not respond to the administration of iron, but is relieved when the milk is supplemented with copper as well. Copper is widely distributed in the animal organism.[179] The central nervous system is richer in its content of this element than is any other tissue, except liver.

metal on the metabolism of iron.[186, 188] Copper appears to influence the absorption of iron from the intestinal tract. Moreover, evidence has been presented which indicates that copper functions in the synthesis of iron and protoporphyrin to form heme, for even when iron is administered intravenously to copper-deficient swine, heme synthesis is unaffected

Extensive skeletal changes have been described in dogs[189, 190] and in swine[191] as a result of copper deficiency. In both species the changes, which we have studied, appear to be similar.

The first alteration noted in the dog appears after two to four months, when the animals begin to develop lameness and deformities of the extremities The forelegs bow outwards at the elbows, while the hind legs bow inward. The wrists and elbows become enlarged. Hyperextension of the wrist joints is prominent Many animals develop obvious fractures, some of which are multiple and appear unrelated to any specific trauma Similar gross changes have been noted in swine

Roentgenological examination of the dogs reveals great thinning of the cortices of all the bones examined Numerous fractures are also encountered In dogs and swine microscopic examination shows no abnormalities in the appearance of the epiphysial or costal cartilages. In the dogs the cartilage

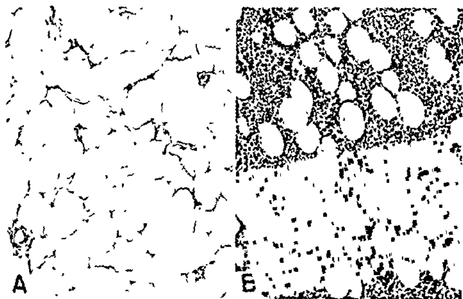


FIGURE 26 COPPER DEFICIENCY

Bone marrow, rib, pig A Normal pig This is composed mainly of fat with a few intervening foci of cellular elements B Copper-deficient pig showing hyperplasia which is due mainly to increase in erythyroid elements H and E (x 180)

philic and polychromatophilic series are present than in the normal animal. The findings in swine are at variance with those reported in dogs in which normocytic and normochromic type of anemia has been observed.[183]

TABLE III

SOME EFFCTS OF COPPER OR IRON DEFICIENCY ON BLOOD, BONE MARROW, AND TISSUE OF SWINE

	Normal	Copper Deficient	Iron Deficient
A *Peripheral Blood*			
R B.C (x10⁶/cu. mm)	7 59	5 59	5 87
Hgb (gm %)	14 0	6 4	5 9
VPRC (ml /100 ml)	42 0	22 0	21 0
MCV (cu μ)	55 0	43 0	36 0
MCHC (%)	33 0	29 0	28 0
Reticulocytes (%)	5 0	4 0	9 4
WBC (x10³/cu mm)	15 3	10 4	16 6
Platelets (10³/cu mm)	520 0	615 0	1311 0
Plasma iron (μg %)	175 0	38 0	30 0
Total iron bind cap (μg %)	51.0	628 0	864 0
Plasma copper (μg %)	186 0	15 0	207 0
B *Bone Marrow—Erythroid cells*			
Diam Nucleus	4 9	6 2	6 5
Diam Cytoplasm	7 6	7 4	7 8
Ratio nucleus-cell	0 64	0 84	0 83
Basophilic (%)	10 0	24 0	29.0
Polychromatic (%)	59 0	70 0	68 0
Orthochromic (%)	31 0	6.0	3 0
Mitoses per 1000 cells	38 0	42.0	6 0
L.E. Ratio	1.77	.59	.82
C *Tissues*			
Copper content (μg /gm)			
Liver	25 0	1 4	59 3
Spleen	1.4	0 5	0 9
Heart	4 0	1.5	4 0
Iron content (μg /gm.)			
Liver	46 0	18 0	7 0
Spleen	12 0	8 0	3 0
Heart	3 0	3 0	2 0

By utilizing the radioactive chromium labeling technique, the life span of the red blood cells in copper-deficient swine was found to be reduced.[187] When red cells from copper-deficient swine are transfused into normal animals, their survival is normal, so, too, normal cells survive for normal intervals when administered to copper-deficient animals. Thus it appears that the anemia observed in this species is due to a shortened erythrocyte survival time, as well as to a reduced capacity of the bone marrow to produce a normal number of red blood cells.

Studies upon copper-deficient swine have shown the importance of this

metal on the metabolism of iron.[186, 188] Copper appears to influence the absorption of iron from the intestinal tract. Moreover, evidence has been presented which indicates that copper functions in the synthesis of iron and protoporphyrin to form heme, for even when iron is administered intravenously to copper-deficient swine, heme synthesis is unaffected.

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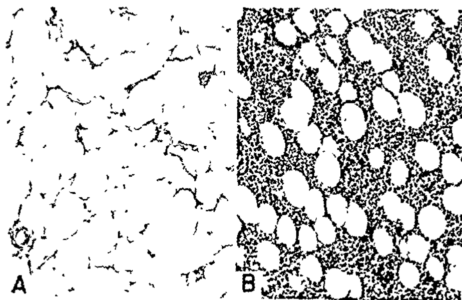


Figure 26 Copper Deficiency

Bone marrow, rib, pig A Normal pig This is composed mainly of fat with a few intervening foci of cellular elements B Copper-deficient pig showing hyperplasia which is due mainly to increase in erythyroid elements H and E (x 160)



B C D

FIGURE 27. COPPER DEFICIENCY.

... left is control Dogs ...how gross deformities. ...nt animal Note thinning of cortex and absence of trabeculae markings. D. Copper deficient dog Bowing, fractures and compression of epiphyses have occurred. (Courtesy of Dr. James Baxter.)

of the deficient group appears to be proliferating even more rapidly than that of the controls. Nor is any change in the deposition of inorganic materials found in the cartilage matrix. However, in the metaphysial regions of both species marked alterations are found. Prominent is a persistence of the spicules of calcified cartilagenous matrix which is devoid of encasing bone. Such a picture develops as a result of a virtual cessation of osteoblastic activity Spindle-shaped cells are present but do not appear to be functionally potent in the sense that osteoid is being produced Thus a zone, or "lattice," of calcified cartilage matrix is found between the epiphyseal cartilages and diaphysis. Such a zone appears to be a weakened area, since fresh fractures and, in particular, healed fracture patterns can be found *When fractures of the calcified cartilage matrix do occur,* osteoblastic activity appears to be stimulated.

In both species the cortex of the long bones is reduced in thickness The process here is difficult to follow because of its slow course No evidence of excessive bone destruction is to be found The rarefaction has been interpreted as being due to normal destructive processes in the presence of decreased bone formation. In neither species is any evidence of rickets pres-



...ICIENCY

Cartil. ... A Normal control Note orderly arrangement of rows of car... and presence of calcified cartilaginous matrix upon which bone is being deposited. *B*, Note normal row formation of cartilage cells with presence of excess lattice of calcified cartilage matrix beneath. H. and E. (x 35).



Figure 27. Copper Deficiency.

Dog *A*. Three littermates after three months on diets. Dog on left is control. Dogs in middle and on right are on copper-deficienct ration, their legs show gross deformities. *B*. X-ray of foreleg of control dog shown in *A*. *C*. Copper-deficient animal. Note thinning of cortex and absence of trabeculae markings. *D*. Copper-deficient dog. Bowing, fractures and compression of epiphyses have occurred. (Courtesy of Dr. James Baxter.)

achromotrichia factors, such as pantothenic acid and para-aminobenzoic acid, the relationship of copper to graying of hair became somewhat confused. The situation has been clarified, however, by experiments which show that the achromotrichia produced by copper deficiency is not affected by large amounts of pantothenic acid; moreover, the fur in animals deficient in copper has a brownish color, contrary to the silvery-gray which develops when pantothenic acid is lacking[192] Achromotrichia has been described in other species deficient in copper: rabbits,[194] dogs[189] and sheep[195] The reaction of copper to the tranformation of tryosine to melanin seems definite enough, though the precise chemical reactions are not known[1508]

The physical nature of hair also appears to be related to copper since in deficient sheep the crimp becomes less distinct and the wool assumes a characterless hair-like structure Studies[1126] have shown that the bio-

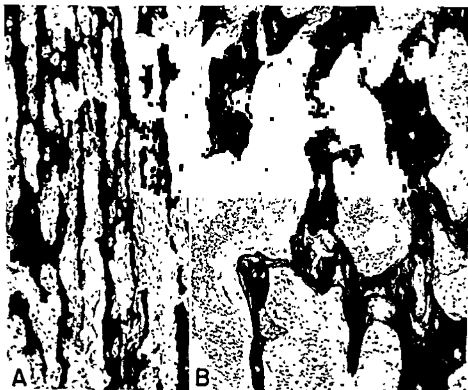


FIGURE 30 COPPER DEFICIENCY

[illegible]

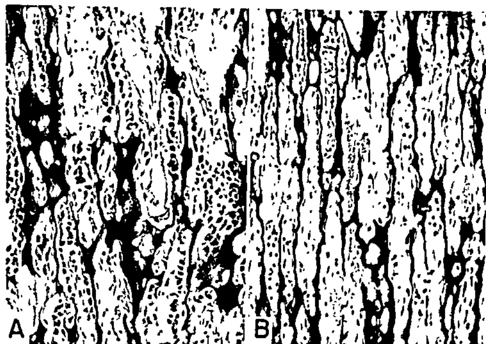


FIGURE 29 COPPER DEFICIENCY.

Metaphysis of pig. *A* Normal lattice of calcified matrix about which are numerous osteoblasts which appear to be promoting osteoid formation. *B*. Lattice from copper-deficient swine to show increased density due to decreased removal, lack of bone deposition and acellularity H and E (x 145).

ent. Chemical analyses of the dog's bones show normal ash and mineral content.

These changes are of great interest for several reasons. In the first place there is a complete dissociation between chondroblastic and osteoblastic function. The former cells continue to proliferate, while the latter virtually cease their activities. In most deficiency states cartilage and bone growth are affected similarly. Copper deficiency is an exception and marks this element as being intimately concerned with the function of the osteoblast and in the production of bone matrix The situation is not unlike that encountered in ascorbic acid deficiency. This similarity will be commented on later (page 178). In view of this interesting effect of copper deficiency on osteoblastic activity, other mesenchymal cells, such as fibroblasts and odontoblasts, must be studied.

Copper appears to play a role in the pigmentation of hair It was first demonstrated that the fur of black-coated rats becomes gray after such animals are placed on a copper-deficient diet.[192] With the discovery of other

cytoplasm of cells. A small amount of iron, approximately 1 mg., is excreted by the adult each day via the bile, intestine and urine Hence virtually all of the iron which is absorbed is retained by the organism.

Few studies have been reported on pathologic changes produced by experimental iron deficiency in animals other than the effects on the blood. Hematological data have been presented in rats,[181] rabbits,[182] dogs[183] and swine[185] All species show a hypochromic, microcytic anemia The mean diameter, mean corpuscular volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration are all reduced (Table III). In advanced stages anisocytosis and poikliocytosis of the red cells are present, so, too, reticulocytes and erythroblasts may be encountered in blood smears The leukocyte count is normal, thrombocythemia may be present. Examination of the bone marrow reveals erythroblastic hyperplasia Erythrocyte protoporphyrin concentration is increased. Serum iron levels, as expected, are decreased As noted in Table III, plasma iron levels in iron-deficient swine may fall from normal levels of 142.7 gamma

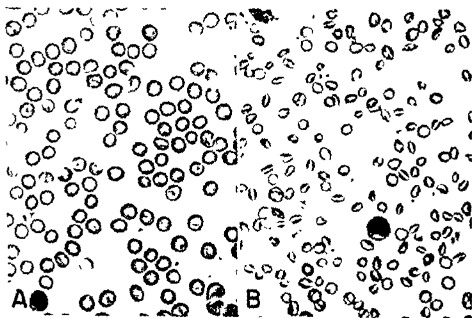


FIGURE 31 IRON DEFICIENCY

Blood smear, rat A. Smear taken before young rat was placed on diet of whole milk supplemented with copper. B. Smear forty days later, at time of sacrifice Note numerous microcytes, achromia and poikilocytosis The blood showed the following values R B C, 4,290,000, Hb, 1.7 gm; MCV, 44 cu.μ, MCHC, 19 per cent, MCH 8 6 $\mu\mu$ Wright stain (x 800).

chemical processes whereby the hair is fixed by oxidation are interfered with, so that, as the fiber is extruded, it remains plastic.

Certain chemical studies are of interest in copper-deficient animals. The cytochrome oxidase activities of liver, myocardium and bone marrow are decreased in rats.[196, 197] Extensive studies of the biochemical defects in copper-deficient rats have been reported recently.[1475, 1476] The activities of liver cytochrome oxidase and succinoxidase are affected early. The decrease in the latter has been ascribed to reduction in the former. Increased isocitric acid dehydrogenase and decreased DPN-cytochrome C reductase activities are found. There is loss of cell preparations to oxidize substrates in the tricarboxylic cycle. Catalase activity is unaffected. These studies should go far to elucidate some of the complex roles of copper in the organism.

IRON

Iron salts are said to have been used by Sydenham during the 18th century for the treatment of chlorosis. The full significance of the role of iron in the organism was not fully appreciated until the element was demonstrated to be an essential component of hemoglobin.

Iron of the food is absorbed from the stomach and duodenum.[198] Certain factors influence its uptake: (1) valence; Fe^{++} is absorbed more readily than Fe^{+++};[199] (2) pH; an alkaline medium decreases absorption;[200] (3) phosphorus and phytic acid; these form complexes which favor non-absorption; (4) presence of copper; absorption is mediated by copper;[186] (5) amount of iron present; the higher the concentration, the greater the absorption [202]

A protein, apoferritin, has been postulated to be formed by the cells of the intestinal mucosa; this governs the absorption of Fe^{++} by combining with it to form ferritin. The iron then enters the circulation as needed and is changed to Fe^{+++}. Such iron is carried in the plasma in combination with a beta-globulin and may be measured. A sensitive index of the state of iron metabolism is thus available. The average human adult contains about 4.5 grams of iron which is found in varying quantities: hemoglobin, 72 9 per cent; myoglobin, 3 3 per cent; cytochrome, catalase, and peroxidase, .2 per cent and storage forms, principally ferritin and hemosiderin, 23 5 per cent.[201] About 27 to 28 mg. of iron are released each day in the adult human from the breakdown of hemoglobin. This iron is used for resynthesis of blood pigment. Iron exists in storage forms in the liver, spleen and bone marrow as the compounds, ferritin and hemosiderin. The latter may be demonstrated by the familiar Prussian blue reaction. Ferritin is more difficult to demonstrate since it is more soluble and diffusely distributed in the

Filmer and Underwood [1129] in cattle and by Marston [1130] in sheep during the period from 1933 to 1935 in Australia One of the most exciting developments in the field of nutrition during the past decade was the discovery that vitamin B_{12} contains cobalt [205]

In view of the presence of cobalt in vitamin B_{12}, it is interesting that this metal had been implicated in erythropoeisis for some time, ever since the Waltners [206] showed in 1929 that polycythemia could be produced in rats when cobalt was fed or administered parenterally The cause for the erythrocytosis is not clear, although fixation of thiol (-SH) groups in the tissues may lead to local anoxia in blood forming tissues [1125] The role of cobalt as part of the vitamin B_{12} molecule is discussed elsewhere (page 277)

Efforts to produce cobalt deficiency in laboratory animals have not been successful Diets have been prepared which contain as little as 6 or 3 micrograms of cobalt per kilo [207] At these low levels of intake no effects appear in rats. Similar studies in rabbits have been negative [208] However, in dogs which have been placed on a milk ration and rendered anemic by bleeding, the administration of cobalt seems to stimulate hematopoiesis in some of the animals [209] Unfortunately in these studies the diets contained appreciable amounts of vitamin B_{12}

The syndromes associated with naturally occurring cobalt deficiency in ruminants will be described on page 301 [1125, 1126]

MANGANESE

The indispensability of manganese for the mammalian organism was shown simultaneously in 1931 by Orent and McCollum [210] in rats and Kemmerer, Elvehjem, and Hart [211] in mice

Manganese is widely distributed in tissues Although there are quantitative variations from one organ to another, the element is particularly abundant in liver [212] Manganese is intimately concerned with the activity of certain enzyme systems For instance, this cation has been shown to be essential for the enzymatic catalysis of isocitric acid to alpha ketoglutaric acid and for certain other oxidative and non-oxidative decarboxylations of di- and tri-carboxylic acids which occur in the Krebs cycle [27, 113]

In the initial experiments described by Orent and McCollum, growth of the young rats was normal The estrus cycles of the deficient females were likewise normal,[214] when such animals were mated with normal males, the usual number of young were produced. However, the females failed to nourish their young When the newborn from manganese-deficient females were placed with normal, lactating animals, a few were raised, all were undersized Divergent results were obtained by Daniels and Ever-



FIGURE 32. IRON DEFICIENCY.

Incisor teeth, rat. *A* Upper and lower pigmented incisors of normal rat. *B* Incisors of rat which had been on a milk diet supplemented with copper for eighty-four days The teeth are pearly in appearance.

per cent to 48 0 gamma per cent. The iron content of the tissues is reduced as well.

As is familiar to all who work with the albino rat, the incisor tooth has an orange color. When this species is placed on an iron-deficient diet, the teeth become pearly white.[203] This is not surprising since the intracellular pigment is an iron containing complex.

In contrast to the findings in copper-deficient animals, the cytochrome oxidase of the bone marrow of those deficient in iron is normal or sometimes even elevated,[197] what role differences in cellularity of the marrow may play in the results of such determinations must be taken into account

No lesions have been described in other tissues of iron-deficient animals save those which might be ascribed to severe anemia, such as atrophy of the central liver cells. No alterations have been observed in the heart or skeletal muscles; this is not unexpected since the organism appears to conserve its myoglobin far more carefully than its hemoglobin.[204]

COBALT

Cobalt deficiency has not yet been produced in laboratory animals. The indispensability of cobalt in the nutrition of ruminants was demonstrated by

Microscopic examination of the nervous tissues has failed to reveal any changes. It has been suggested that a chemical lesion may be present.

ZINC

The indispensability of zinc for Mammalia was first shown by Todd, Elvehjem, and Hart[226] in 1934; the rats which they studied exhibited a disturbance of growth which was accompanied by alopecia.

During recent years zinc has come to be recognized as an important constituent of a number of enzymes[21] These include carbonic anhydrase, dehydropeptidase, carboxypeptidase, alcohol dehydrogenase, glutamic dehydrogenase and lactic dehydrogenase. Thus zinc ranks with iron, copper and molybdenum as a trace element which is a specific and integral component of certain enzymes

The zinc content of the tissues of some animals has been reported.[227, 228] Studies which utilized radioactive Zn^{65} in rats have shown that the element is mainly excreted into the gastrointestinal tract by the pancreatic juice.

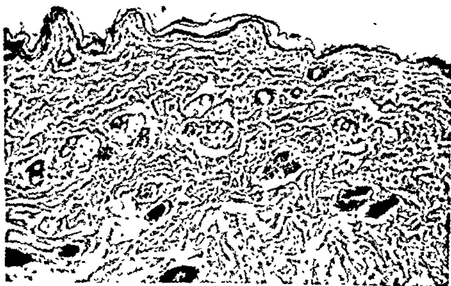


FIGURE 33 NORMAL SKIN, RAT

Note epithelium and underlying corium with hair follicles and sebaceous glands The epithelium is several cells in thickness. There is some keratinization although this is not marked. The sebaceous glands are not large. H. and E. (x 150).

son[215] who employed a somewhat different diet from that used by the Baltimore workers. Almost one-half of the young born to manganese-deficient females on this diet were dead or died within a few hours after birth. Such females were able to nourish and rear foster young, while only a few of their own deficient young could be raised by foster mothers. The fault thus appeared to be in the young and not in the mother, an hypothesis which has been strengthened by Shils and McCollum,[216] who noted no disturbance in the estrous cycle of the rat. The University of Wisconsin group had described estrous changes in mice.[211]

Using a somewhat different diet Boyer *et al.*[217] have shown that manganese is necessary for growth in the rat, an observation which has been confirmed by Shils and McCollum.[216] The latter investigators have also called attention to another specific feature of the manganese deficiency syndrome. Some of the young of manganese-deficient females exhibit ataxia, incoordination and loss of equilibrium; they tend to fall over and have difficulty in righting themselves. The underlying cause of these phenomena has not yet been elucidated.

Sterility of male rats reared on manganese-low diets has been a constant finding. Microscopic examination reveals absence of spermatogenesis,[217] whether this change may be a result of inanition is a point which has not been specifically investigated Testicular changes have not been observed in rabbits[218]

In both rats and rabbits the development of skeletal abnormalities has been associated with low manganese rations. In the former species shortening of the tibia has been described,[219] while in another study bowing of the forelegs has been noted.[216] Gross inspection of the sectioned bones of manganese-deficient rats has revealed an absence of trabeculae in the metaphyseal regions.[220] In all of these studies the change has occurred only in the young from manganese-deficient females In contrast, skeletal changes are easier to produce in growing rabbits[221,222] Rarefaction of the bone with bowing is prominent. No fractures appear to have been noted. On microscopic examination, such bones appear rarefied, particularly in the metaphyseal regions. In swine, lameness and enlargement of the hock joints have been ascribed to manganese deficiency; the changes could be prevented by adding manganese to what appeared to be an adequate diet.[223]

Further studies in swine[224] have indicated shorter, broader legs in animals deficient in manganese. General rarefaction was also described. More studies will have to be undertaken before the exact pathogenesis of these alterations is understood.

When families of rats are raised on a manganese-deficient ration, several animals of the second generation have developed ataxia and disturbance in equilibrium.[225] These signs appear earlier in each succeeding generation.

Microscopic examination of the nervous tissues has failed to reveal any changes. It has been suggested that a chemical lesion may be present.

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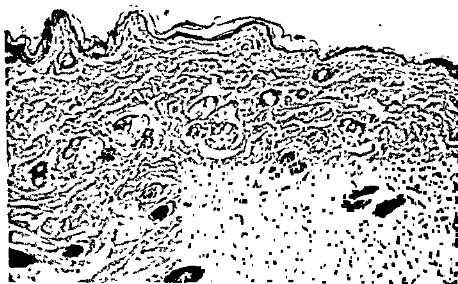


FIGURE 33 NORMAL SKIN, RAT

Note epithelium and underlying corium with hair follicles and sebaceous glands The epithelium is several cells in thickness. There is some keratinization although this is not marked. The sebaceous glands are not large. H. and E. (x 150).

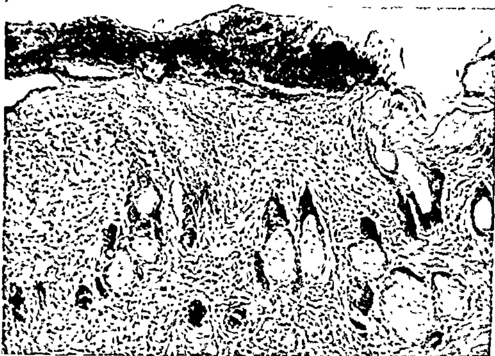


FIGURE 34 ZINC DEFICIENCY.

Skin, rat Note keratinized cells overlying a layer of thickened epithelium which shows acanthosis There is atrophy of the hair follicles with persistence of the sebaceous glands, in fact these structures are hypertrophied in comparison with the normal This animal had been on a zinc-deficient diet for seventy-four days H. and E. (x 150).

When injected, it disappears rapidly from the plasma and soon appears in red blood cells and liver. The most active turnover is found in the liver, pancreas, kidney, and pituitary, the least active turnover is in the red blood cells, brain skeletal muscles and skin.[229, 230, 231, 238]

Todd *et al.*,[226] who employed a diet which contained only 1.6 parts zinc per million, noted a disturbance in growth and alopecia in rats, both abnormal manifestations could be prevented by supplementing the diet with about 100 μ gm of zinc each day

Day and McCollum[232] devised a ration which furnished only 1 to 4 μ gm of zinc daily Zinc was removed by extraction of the dietary components with diphenylthiocarbazone, a laborious process. The control diet furnished .15 mg. of zinc daily. When young rats were placed on the zinc-deficient regimen, they ceased gaining weight in two to three weeks. Death occurred as early as the thirty-third day of the deficiency. Thinning of the hair became apparent after the third week and was followed by alopecia

over portions of the dorsum of the body The denuded areas were roughened and scaly.

The histological changes in these zinc-deficient animals were studied by the present writer in association with Day and McCollum.[231] Microscopically, the skin showed hyperkeratinization and acanthosis The epithelium increased from the normal of three to four cells in thickness to eight or ten cell layers. On the surface an increased number of completely or partially keratinized cells was found No appreciable acceleration in mitotic activity of the basal cells could be discerned, although quantitative measurements were not performed Clear spaces appeared in the cytoplasm of many cells, the nuclei of such cells became pyknotic In advanced cases the skin was covered by a crust of keratinized debris, bacteria and leukocytes, the corium underlying such areas was hyperemic and infiltrated with white blood cells. Accompanying such epidermal changes, there was atrophy of the hair follicles, which ultimately disappeared, leaving only a few mononuclear cells to mark where they had been. The sebaceous glands remained



FIGURE 35 ZINC DEFICIENCY

Esophagus, rat A Cross section of normal B Esophagus from rat which had been on a zinc-deficient diet for seventy-four days. Note increase in thickness of the lining epithelium. In comparison with normal, there is extensive parakeratosis, together with an increase in thickness of the basal cell layer. H. and E. (x 50).



FIGURE 36. ZINC DEFICIENCY.

Esophagus, rat A. Normal. B Zinc-deficient rat shown in Figure 34 Note wide zone of partially keratinized cells, many of which retain their nuclei H. and E. (x 135).

intact, even late in the deficiency the cells making up these structures were larger than those of the controls. Skin from the tail, ears, and paws showed no abnormality.

Extensive changes were found in the esophagus, such alterations consisted of an increase in thickness of the epithelial lining together with the appearance of large, partially keratinized cells on the surface. The normal esophageal epithelium of the rat is made up of a basal stratum of cells, some of which exhibit mitotic figures. Above this there are several layers of larger cells with clear nuclei whose cell borders are indistinct. From these an abrupt transition to a keratinized layer occurs. The basal cells of the zinc-deficient rat were more numerous and closely packed; the overlying stratum was six to eight cells thick. Along its innermost edge the nuclei became pyknotic but did not disappear in normal fashion, so that here a zone of partially keratinized cells, ten or twelve cells in thickness, was found. The submucosa was normal Similar alterations were found in foci on the posterior portions of the tongue and the posterior roof of the mouth. The fore stomach was not involved. The change was interpreted as being due either to a retardation in normal keratinization, or to an increased proliferation of cells This lesion in the esophagus is unique. Among the many

studies of dietary deficiences, similar changes have not been recorded in this structure.

In two zinc-deficient animals vascularization of the cornea was observed. This consisted of an ingrowth of capillaries into the tunica propria and infiltration of this tissue by leukocytes. There was no keratinization of the epithelium, the lacrimal glands were normal In view of the occurrence of corneal vascularization in other deficient states, it cannot be classed as a specific abnormality (page 454) No changes have been found in any other tissues from such animals save those which must be ascribed to inanition.

Studies of the zinc content of the bones of these rats revealed a reduction to 94.7 micrograms per gram of ash in the deficient animals, as compared with a value of 236 6 micrograms in the controls[232]

Measurements of the carbonic anhydrase activity of the red blood cells obtained from zinc-deficient animals have indicated no change from normal[232]

Zinc deficiency has also been produced in mice[234] This species develops alopecia Microscopic examination of the skin reveals changes similar to those which were described above in rats.[235]

An interesting disturbance, parakeratosis disease, has been recognized in swine for a number of years.[236] The syndrome is characterized by failure to grow, skin lesions, diarrhea, vomiting, anorexia and death. When the skin is examined microscopically, extreme parakeratosis is found, this resembles the change which has been described in zinc-deficient rats and mice. These signs and symptoms in swine may be prevented by zinc administra-

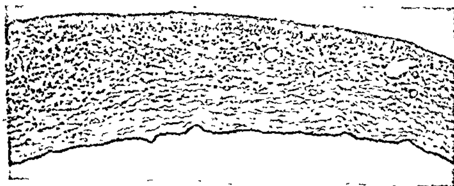


FIGURE 37. ZINC DEFICIENCY

Cornea, rat Section from an animal which had been on a zinc-low diet for seventy-four days. Note several large vessels, infiltration with leukocytes, and thickening of the epithelium. H. and E. (x 60).

tion. It is of interest that the requirement of swine for this element is higher (34 to 44 mg. per cent of the ration) than it is for rats and mice[237]

IODINE

Iodine was discovered accidentally in 1811 because of its corroding action on kelp vats in which the decomposition of calcium nitrate was being carried out. Its importance for the organism was shown in 1896 when Baumann[239] found a high concentration of iodine in thyroid tissue Twenty years later, Kendall[240] demonstrated its presence in thyroxin, an active principle of the thyroid gland. A good deal of our present knowledge of iodine is derived from studies of certain disease states which occur naturally in man and animals These are detailed in the section, "Endemic Goiter" (page 307).

Ingested iodide is absorbed into the blood stream from the intestinal tract. In health almost all of the circulating iodide is removed by the thyroid epithelial cells, a little is withdrawn by salivary gland epithelium and gastric mucosa. Traces are excreted in the urine. The mechanism whereby iodine is taken up or "trapped" by the thyroid cells is not known. Intact cells are necessary whether the process is studied *in vivo* or *in vitro*.[241] Iodine uptake by the thyroid is inhibited by thiocyanate (SCN) and certain other molecules.[242] Iodine atoms are rapidly incorporated into the tyrosine molecule to form first, monoiodotyrosine, then, di-iodotyrosine. By reactions not yet understood, two molecules of di-iodotyrosine are united to form tetra-iodotyrosine or thyroxine. The incorporation of inorganic iodine (as free iodine) into the organic molecule of tyrosine is blocked by certain "antithyroid" compounds which contain either aminobenzene ($NH_2C_6H_5$) or thiourea NH_2-CS-NH_2) groupings.[243, 244] The thiourea series of compounds appear to inhibit the activity of the enzyme, peroxidase, which may be of importance in the transformation of inorganic iodine to organic compounds.[245] The discovery of sulfaguanidine as the first of the "antithyroid" compounds by the Mackenzies and McCollum in 1941[246] has truly revolutionized thyroidology. So, too, the availability of radioactive iodine (I^{131})-with which to study normal and diseased thyroid glands has yielded much information.

The exact biochemical transformations which take place in the thyroid epithelial cells during the elaboration of thyroid hormone are not known. However, a protein, thyroglobulin, with a characteristic amino acid composition may be isolated from the gland This protein contains iodo-tyrosines and thyronines It appears to be formed initially within the cytoplasm of the epithelial cells and then to be extruded into the lumen of the follicle,

probably becoming incorporated into the colloid of this structure Upon the proper stimulus, ordinarily provided by hypophyseal TSH, thyroglobulin is broken down with the liberation of thyroxine into the circulation Until recently, tetra-iodotyrosine (thyroxine) was the most active preparation known, although it was generally thought not to be *the* active principle, since an appreciable time is required for it to exhibit its action. Moreover thyroxine is not active *in vitro*. Recently, tri-iodotyrosine has been isolated in the gland and in blood serum. This compound is many times more potent than thyroxine. Whether it represents the long sought active principle remains to be determined.[247] The metabolic transformations of iodine in the thyroid gland are summarized in Figure 38.

Before discussing the pathological effects resulting from relative or absolute iodine deficiency, mention must be made of current concepts of thyroid regulation and of the many extraneous factors which affect the gland, all of which must be borne in mind when iodine deficiency is being studied. That there is a reciprocal relationship between the pituitary and thyroid glands seems well-established.[248, 1174] When, for some reason, the concentration of thyroid active principle in the blood falls, an increased production of pituitary thyrotrophic hormone (TSH) results Conversely, an increase

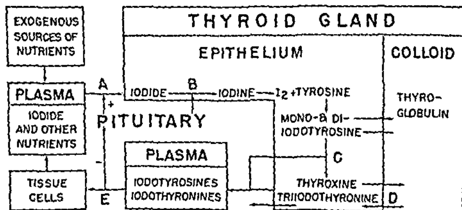


FIGURE 38 IODINE METABOLISM IN THE THYROID GLAND.

A schematic representation of the metabolism of iodine in the thyroid gland together with various factors which may affect it, including the pituitary gland and certain chemical compounds A Iodine trapping mechanism which is inhibited by SCN, ClO_4 and other compounds[241, 242] and [illegible] ...odide to iodine which is inhibited by [illegible] ...y the pituitary C Coupling reaction [illegible] ...otease reaction which liberates thyroni[illegible] ...onines and action of active principle o[illegible] TSH is formed.

in thyroid active principle suppresses pituitary TSH elaboration. Since the hypophysis appears to be the controlling factor in this dual relationship, two groups of possible conditions, which are based on pituitary activity, may be considered: (a) those in which TSH is absent or reduced, and (b) those in which this hormone is increased. When the former conditions prevail, thyroid *hypoplasia* is to be expected; thyroid *hyperplasia* will usually occur following the latter situation. Morphologic thyroid hyperplasia does not necessarily imply clinical hyperthyroidism associated with an increased basal metabolic rate. On the contrary, it is important to realize that body metabolism, as measured by oxygen uptake, may be elevated, normal, or depressed in the presence of morphologically hyperactive thyroid epithelium. Table IV summarizes some of the various known and hypothetical factors which may lead to the conditions so briefly alluded to above.

TABLE IV

PITUITARY-THYROID RELATIONSHIPS

I *Pituitary Stimulus Reduced or Absent (Thyroid Hypoplasia)*
- A Hypophysectomy[243, 249]
- B. Increased blood concentration of thyroid active principle
  - (1) Increased administration[245]
  - (2) Increased production (?)
  - (3) Decreased need
    - (a) Starvation, inanition[11]

II *Pituitary Stimulus Increased (Thyroid Hyperplasia)*
- A Primary pituitary hyperfunction[1174]
- B TSH administration[250]
- C. Decreased thyroid hormone production due to.
  - (1) Removal of thyroid tissue[255, 1477]
  - (2) Interference with thyroid cell function.
    - (a) Iodine deficiency in diet[251, 252, 258, 259]
    - (b) Mineral imbalance. calcium,[253] sodium chloride,[254] other (?)
    - (c) Amino acid deficiency phenylalanine, other (?)
    - (d) Vitamin deficiency (co-enzymes, etc.) (?)
    - (e) Impaired iodine uptake SCN[242]
    - (f) Impaired iodine utilization by antithyroid compounds[243, 244]
    - (g) Interference with release of thyroid active materials (?)
  - (3) Increased utilization
    - (a) Cold[250]

Studies of experimental iodine deficiency have been intimately concerned with the problem of goiter, a term which is used much too loosely. Goiter means an enlargement or increase in weight of the thyroid gland. Two types of goiter are recognized. One is due to an abnormal deposition of colloid material in the follicles of the gland; this is colloid or simple goiter. A second form is due to hyperplasia of the epithelium of the follicles; this is hyperplastic goiter, which may or may not be accompanied by clinical

hyperthyroidism It is unfortunate that the term goiter is used so frequently without a precise qualification One should always determine whether a thyroid gland is enlarged as a result of excessive colloid deposition, because of epithelial hyperplasia, or for some other less common cause, such as neoplasm or inflammation.

Modern studies of experimental goiter began when Halsted[255] extripated almost all of the thyroid tissue from dogs early in pregnancy and found glandular hyperplasia in their offspring It was soon demonstrated that such canine "congenital goiters" could be prevented by the administration of potassium iodide to the female during gestation.[256] Further interest was aroused when it was shown that the iodine treatment of sows from certain areas prevented the birth of hairless, cretin-like offspring [257] Such

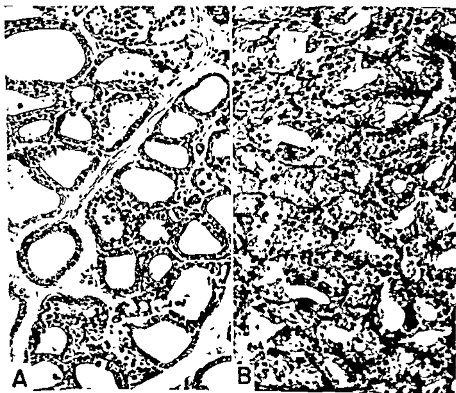


FIGURE 39 IODINE DEFICIENCY. [illegible]

[illegible]

experiments lent support to the growing idea that iodine deficiency and naturally occurring goiter were closely related (see page 310). The production of experimental goiter on the basis of iodine deficiency alone has been difficult to realize, however. This is because any experiment which is designed to study thyroid structure in relation to iodine deficiency must take into account many diverse factors, such as those noted in Table IV.

That absolute or relative iodine deficiency leads to an hyperplastic thyroid gland seems well-established in the experimental animal.[251, 252, 254, 259] In the rat various degrees of gross thyroid enlargement have been produced. Depending, of course, on certain variables such as the composition of the diet and its iodine content, the initial alteration is found in the epithelium. The cells change from their flat or cuboidal forms into columnar structures; intra-cellular vacuoles may be observed. No pointed study has been made of the size of the nuclei, or of any other intracellular constituent. Hyperplasia leads to infolding and partial obliteration of the lumens of the follicles, accompanied by a great reduction in colloid. Necrosis of the epithelium has been observed, and may be related to the iodine content of the ration, for the lower the iodine concentration, the more extensive the necrosis. In addition to these cellular changes, the gland appears to increase in vascularity. No lymphocytic infiltrations, which are so characteristic in the naturally occurring hyperplastic gland in the human (page 313), have been described in experimental animals. It must be emphasized that the morphological picture thus far produced experimentally by iodine deficiency is that of hyperplastic goiter, not colloid goiter, at least as the latter is seen in man (page 307). To our knowledge, colloid goiter has not been produced in the laboratory in the form in which it occurs naturally.

Before the advent of the various goitrogenic anti-thyroid compounds in the early 1940's it was clear that certain factors affected the production of hyperplastic alterations in the thyroid glands of rats on so-called "iodine deficient diets." For instance, the presence of excess calcium[253] appeared to increase hyperplasia, on the other hand casein in the diet seemed to inhibit any thyroid change.[260] After the dramatic effects of such hyperplastic goitrogens as thiocyanate, sulfaguanidine and the thiourea compounds had been reported,[243, 244] it was only natural to wonder whether any goitrogens had been present in the "iodine deficient diets" hitherto used. So far, such have not been observed. For instance, when mice were placed on Remington's[254] standard low iodine diet, the removal of its individual components: cornmeal, yeast, or wheat gluten, failed to affect its goitrogenicity. Surprisingly, the only component of the diet which appeared to influence the size of the thyroid gland was sodium chloride, whose omission greatly reduced the size of the goiters, though thyroid hyperplasia was present even in its absence. In another study[259] goiter was produced

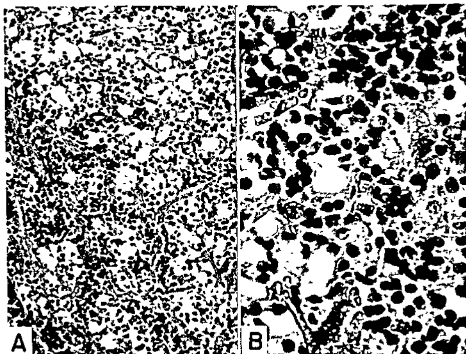


FIGURE 40 IODINE DEFICIENCY

Pituitary gland, rat *A* Low magnification (x 200) and *B* Higher power (x 575) of pituitary from animal which had been on iodine-deficient diet and whose thyroid gland was hyperplastic The cells which special stains show to be basophiles are enlarged with vacuolated cytoplasm, i e, they are so-called thyroidectomy cells [243]

in rats on a diet which was as free of iodine as possible since the foodstuffs comprising the ration were raised by the hydroponics technique

The pituitary gland of the iodine-deficient animal shows characteristic changes associated with the hypothyroid state [243, 1477] The so-called basophilic thyrotropes increase in size and number Some become vacuolated. As a result the size of the gland increases.

FLUORINE

The presence of fluorine in foodstuffs and, as a result, in the mammalian organism has been recognized for sometime. This element assumes great importance today because of the relation of the fluoride content of natural water supplies to the incidence and severity of dental caries Hence, numerous programs aimed at a reduction of the prevalence of dental caries

by the addition of small amounts of fluoride to water supplies are in effect. In the United States more than thirty million persons now drink fluoridated water. The problems entailed in such an extensive public health program cannot be taken up here. Reviews are available.[262, 276] As might be expected this program has led to many publications with claims that countless diseases, ranging from heart, arterial, kidney, stomach ailments to mental degeneration are being produced. It is purported to "speed up cancer processes." Moreover, fluoridation is cited as a technique in mass control through medication, which is an "integral part of Communist philosophy."[277]

Most foods contain .2 to .3 parts fluorine per million[262] Hence, one would not expect the body content to be particularly high unless some other source is available. That this is so as a result of high levels in naturally occurring water supplies is well-known. Levels may vary from 1.0 p p m up to 20 p.p.m. The importance of fluoride in producing alterations in the teeth was derived from studies on mottled enamel, a syndrome described by McKay in 1916.[263] In 1931, it was conclusively shown that changes in the enamel of the teeth may be found when there is an increased concentration of fluorine in the drinking water.[264] Such lesions, mottled enamel or enamel hypoplasia, consist of pits and depressions in the enamel covering of the teeth. Since 1931, numerous epidemiological studies have proved that this disease is related to the fluoride content of drinking water and that, as the concentrations of fluoride increase, the severity of mottled enamel likewise becomes more severe. The disease is particularly prominent in this country, especially in New Mexico and certain portions of Texas, where the fluoride content of the water may be very high. For instance, in Lubbock, Texas, where the water contained 4 4 parts of fluorine per million, over 90 per cent of the children between the ages of nine and eleven had mottled enamel.[265]

As would be expected from the gross appearance, histological examination of such teeth reveals that the ameloblasts are most affected;[266] such cells apparently become inactive and assume a flat shape. In addition, defects in calcification of the dentine appear and this material becomes stratified. There is also damage to the odontoblasts, many of which have pyknotic nuclei. Though only the teeth of the growing organism are affected, bone may be involved in hyperfluorosis at any age; such osseous changes consist of thickening of the trabeculae in the shaft and periosteal new bone formation about the cortex.[266] In the human, the concentrations of fluoride in the drinking water necessary to produce these changes must be relatively large and a fairly long period of ingestion of such concentrations must prevail before radiological and histological defects can be found in the bones.[267]

As already noted, the most important phase of fluorine metabolism in

its relation to preventive medicine is the accumulating evidence that certain concentrations of fluoride compounds in drinking water protect against the development of dental caries. Several years ago because the water supply in Bauxite, Arkansas, contained such a high content of fluorine the source of the water was changed to one which contained virtually none of this element[268] About ten years after this change the teeth of the children in Bauxite were examined; the incidence of mottled enamel was found to be as high as expected; more interesting, however, was the observation that these children who had been drinking such fluorine-free water for ten years had an extremely low incidence of dental caries[269] In contrast, children living in a near-by town who had used the same source of fluorine-free water all their lives had a high incidence of caries.

Chemical analyses of the fluorine content of carious teeth have shown decreased amounts of this element when comparison is made with sound teeth.[270]

Epidemiological surveys on the incidence of caries in relation to fluoride content of the drinking water and other factors have helped clarify our understanding of the problem[271, 272] So, too, the topical application of sodium fluoride in various communities which have been well controlled by observations in other areas clearly shows the effectiveness of this form of prophylaxis against dental caries[273, 274]

These observations have stimulated the initiation of fluoridation programs in any number of communities in the United States during the past decade and more recently in other parts of the world Currently it is estimated that over 30 million persons are drinking fluoride treated water More on fluoridation will be found in the bibliography[262, 275, 276, 277] and in the section, *Dental Caries*, on page 439

The other side of the story, deliberately produced fluorine deficiency, is in need of much further study. Only a few attempts have been made to produce fluorine deficiency in experimental animals Some years ago Sharpless and McCollum[278] fed three generations of rats a diet low in fluorine content, no effects were noted So too, Evans and Phillips[279] fed rats a milk diet for five generations and found only a decrease in the fluoride content of the skeleton McClendon[280] has utilized the hydroponics technique to prepare fluorine-free foodstuffs and has studied the effects of feeding such materials to rats. One animal died in forty-eight days of starvation, because severe caries prevented chewing, while a second rat lived a little longer but evidenced severe caries at autopsy. Lesions of the teeth have been observed in rats born to mothers which had been placed on rations grown from fluorine-free media Histological studies of such teeth have not been reported. The few animals reported by McClendon furnish the only evidence that fluorine-deficient diets composed of natural foods have any effect on the tooth structure of the growing animal.

MOLYBDENUM

Small amounts of molybdenum were detected in plant and animal tissues a number of years ago [281] The possible importance of this element in nutrition came from the recognition that a disease of grazing cattle known as "teart" was due to excessive amounts of molybdenum in the herbage which they were eating.[282] The similarities of the symptoms of "teart" to those associated with copper deficiency in cattle suggested that the latter element might be of use in treating the disease. This proved to be the case.[283] Moreover, the opposite situation was shown to occur, i.e., molybdenum prevented the detrimental effects of excess copper in the diet.[284]

The relation of molybdenum to at least one enzyme, xanthine oxidase, in mammalian tissues, would appear to place this element in the indispensable group. The concentration of molybdenum in the diet has been shown to affect the activity of xanthine oxidase in certain tissues. This enzyme, which is a flavo-protein, has definitely been shown to contain molybdenum as an integral part of its molecule.[285, 286, 287]

Molybdenum-deficient diets have been fed to rats by the Wisconsin group [288, 289] No effects were noted even when the molybdenum content of the ration reached 01 p.p.m. of dried diet or a daily consumption of 0.5 micrograms per rat. That the intake of molybdenum must be lower than this if biochemical evidence of deficiency (xanthine oxidase activity) is to be detected has been demonstrated by showing that diets containing .02 p p.m. or .2 to .3 micrograms per rat per day are sufficient to produce a saturation level of xanthine oxidase in the tissues [287] Hence, it would appear that diets of lower molybdenum content than those heretofore utilized must be fed to experimental animals if changes other than biochemical ones are to be produced.

Toxic effects of molybdenum have been studied in laboratory animals In rats,[290, 291] growth failure, loss of weight and anorexia appear to be the most prominent effects No anemia has been observed. The inclusion of copper in the diet in overcoming the toxic effects of molybdenum has been demonstrated in such animals. Moreover, methionine has been shown to be equally, if not more, effective.[292, 293]

The inclusion of .1 or more per cent molybdenum in the diets of rabbits leads to a syndrome characterized by anorexia, loss of weight, loss of hair from a dry, scaly epidermis and anemia.[294] No achromotrichia or diarrhea was observed. An interesting deformity of the front legs developed; this was characterized by an outward spreading of the limbs. The pathogenesis of this change is not clear at this time.

Part III
Proteins and Amino Acids

Part III

PROTEINS AND AMINO ACIDS

PROTEIN DEFICIENCY IN GENERAL

Of the three principal foodstuffs. proteins, fats, and carbohydrates, the first are the most important. Besides contributing to the internal framework of the cell and to the structure of such intercellular substances as osteoid, collagen and dentine, proteins furnish the amino acids from which enzymes, hormones, hemoglobins, plasma proteins, antibodies, and many other physiologically active substances are made

E V McCollum[300] and W. C. Rose[301] have traced the development of our knowledge of protein in nutrition One of the first experiments was that of Magendie who in 1816 restricted a dog to a diet of gelatin and found the animal failed nutritionally. In 1848, Mulder coined the term protein Gelatin was often used as a foodstuff, but not until 1905 was it found to support growth unless supplemented with the amino acids tyrosine, cystine and tryptophan At the same time Hopkins had shown that zein may be partially supplemented by adding tryptophan[302] Osborne and Mendel[303] completed the story by adding lysine to tryptophan and zein and so obtained good growth. These experiments initiated the modern investigations of amino acids in nutrition, a subject which W C Rose and his collaborators have done so much to elucidate.

It seems unnecessary to go into detail concerning the metabolism of proteins and amino acids. However, a fundamental concept needs to be stressed One must be concerned not only with the *quantity* but also with the *quality* of dietary protein. For man, the daily requirement of 1 gm of good quality protein per kilo. of body weight has been set. The diet of the growing laboratory animal (rat, mouse, rabbit) is usually fed *ad libitum*, its protein content ordinarily comprises 18 to 20 per cent. As just noted, protein may be administered in sufficient quantity yet be lacking in quality. Certain widely available natural foodstuffs, such as corn and rice, fall into this category and give rise to world-wide qualitative protein malnutrition. Corn and rice are discussed elsewhere with respect to kwashiorkor (page 334), pellagra (page 316) and beriberi (page 406) It is apparent that imbalances of amino acids in natural proteins may lead to nutritional disease syndromes, or at least to disturbances in growth.[26] This phase needs much investigation.

Dietary protein is broken down in the stomach and intestine into its constituent amino acids These together with those derived from the various gastrointestinal secretions are absorbed by the cells of the intestinal mucosa

and carried off by the circulatory system to other areas, particularly to the liver. The amino acids may be grouped into several categories as to how they are to be utilized, i.e., via the three main end-products of metabolism: acetyl CoA, oxaloacetate and alpha-ketoglutarate. These transformations will be found summarized in Figure 45. Certain fundamental metabolic mechanisms, such as deamination and transamination are concerned here.

Before going on to discuss deficiencies of the individual amino acids, protein deprivation in general must be considered, for it is clear that the usual effects of most single or multiple amino acid deficiency states are the result of general protein deprivation. If each of the necessary amino acids is not furnished, no protein at all will be synthesized. A number of important effects may be produced in the organism by restricting dietary protein intake. All of these consequences can be classed as non-specific; moreover, they are common to deficiencies of many of the essential amino acids.

As might be expected, growth of the protein-depleted organism may be greatly slowed.[304, 305] When one examines the skeletal tissues, profound alterations may be encountered.[306] Depending on the severity of the deficient state, varying degrees of retardation of epiphyseal or costal cartilage cell proliferation will be found. Cartilage, as is pointed out elsewhere (page 11), is a critical index of the nutritive status of the organism. Disturbance in protein nutrition leads to a prompt decrease in proliferative activity of its cells. The alteration is entirely non-specific and differs in no wise from the effects of partial or total caloric restriction. Similarly, protein deficiency interferes with osteoblastic activity so that periosteal and endosteal bone formation is retarded, hence, an osteoporotic structure results. Here again the picture is non-specific, resembling that seen in partial or total starvation. Skeletal alterations resulting from protein deficiency are reminiscent of those which follow hypophysectomy. Administration of growth hormone to protein-deficient rats has no beneficial effect on the activity of cartilage cells or of osteoblasts.[307] Protein deficiency in the adult rat leads to rarefaction of the skeleton.[308] Alterations in the teeth and peridontal tissues of protein-starved rats have been described.[309, 310]

The thymus and lymphoid tissues in general are affected by protein deprivation. Atrophy is the rule. The thymus, lymph nodes, and spleen are reduced in size; proliferative activity ceases.

Certain endocrine glands: pituitary, adrenals, and gonads, suffer in a non-specific fashion.

Protein deficiency leads to decreased formation of hemoglobin, red blood cells,[312, 313] and, in particular, plasma proteins.[314, 315] The hypoproteinemia may or may not be accompanied by edema. Some amino acids are more potent than others in correcting these defects.[316]

As might be expected, a good many of the disturbances in plasma protein formation can be ascribed to malfunction of the liver. It has been known for a number of years that hepatic damage follows the ingestion of diets low in protein content. For instance, when rats are placed on a low protein diet the total protein content of the liver falls rapidly so that a large proportion of cytoplasmic nitrogenous constituents may be depleted in only a few days.[317] Functional aspects of this in protein-depleted animals may be brought out by studying the *in vitro* activities of certain enzymes in the livers from depleted animals and comparing these with similar enzyme systems from normal animals.[318] For instance, the activities of pyruvic oxidase, succinoxidase, succinic acid dehydrogenase, d-amino oxidase, DPN-cytochrome C reductase and uricase are reduced. On the other hand the activities of certain other enzymes are unchanged.

The effects of protein deficiency on reproduction in the rat has been studied in some detail.[295, 311] A critical level of about 5 per cent dietary protein was found to insure reproductive function.

Studies of the concentrations of nucleic acid and phospholipid in intact



FIGURE 41. PROTEIN DEFICIENCY.

on a diet adequate
start. *B*, *C*, *D*, and
H. and E

and regenerating nerves of protein-depleted rats indicate no differences in these constituents[346]

Protein deprivation would appear to have a profound effect on the integrity of the cornea and lens. During the early studies of tissue changes associated with nutritional deficiencies, great interest centered upon the eye when corneal vascularization was found in riboflavin-deficient rats[192] Ingrowth of capillaries into the cornea was regarded as a manifestation of riboflavin deficiency in man. As time has elapsed vascularization has been observed to accompany other deficient states, particularly those associated with a lack of protein in general or of single amino acids. Ingrowth of capillaries into the rat's cornea has now been observed as a result of deficiency of each of the 10 essential amino acids.[320]

Cataract may develop during the course of deficiencies of protein or of certain vitamins (page 212). The lens is likewise affected by animo acid deprivation, though to varying degrees[319] Tryptophan, phenylalanine and histidine deficiencies lead to the most marked changes, while less damage is evidenced with a lack of leucine and threonine. Haziness of the lens has been described to result from deficiencies of lysine, isoleucine, methionine and valine.

The role of dietary protein in modifying the response of host cells to those intracellular agents of disease, the viruses, has received much attention.[321] For instance, when the virus of swine influenza was inoculated into mice, the protein nutrition of the latter was of importance in determining the outcome, which has a cyclic variability. Initially, there is increased susceptibility, followed by a phase of increased resistance, and a final stage of increased susceptibility. It is postulated that these three phases correspond to: (1) utilization of the animal's depot fat and carbohydrate reserves, (2) protein reserves, and (3) cells themselves Antibody formation is impaired in protein-deficient animals.[322]

Of the twenty-odd amino acids which have been isolated, Rose has designated ten as *indispensable* for the rat, that is, those which are needed for normal growth. tryptophan, lysine, histidine, arginine, phenylalanine, isoleucine, leucine, threonine, methionine, and valine. Further studies have shown that these same amino acids are necessary for normal growth of the dog and pig and that all but two, arginine and histidine, are necessary to promote a positive nitrogen balance in man. Arginine is synthesized in moderate quantities by the adult rat so that it is only indispensable for the activities of the growing animal

The indispensability of various amino acids has been studied in rats,[323] mice,[324] dogs,[325, 326] swine[327] and man. The influence of amino acids on growth of cells in tissue culture has been reported.[465] In addition to the ten indispensable ones already noted above, cystine, tyrosine, and gluta-

mine appear indispensable for a number of mammalian cell strains which have been studied.

Certain amino acids might be classed as partially indispensable. In this category are cystine and tyrosine which will respectively replace in part the truly indispensable amino acids, methionine and phenylalanine[380, 421]

Some effects of other nutrients on the metabolism of protein must be mentioned For instance, the fibrous protein, collagen, which forms the basic framework of the skeleton, teeth, and connective tissues of all organs, is dependent on normal concentrations of ascorbic acid in certain organisms Another protein, keratin, is affected by disturbances in the concentration of certain vitamins Transamination and deamination reactions require pyridoxal phosphate. So, too, the metabolism of specific amino acids is affected by certain vitamins· tryptophan by pyridoxine, glycine by folacin; the aromatic amino acids by ascorbic acid; et cetera.

TRYPTOPHAN

One of the monuments in the history of nutrition is the observation of Willcock and Hopkins in 1906 that mice fail to grow, and may even die, if the sole source of dietary protein is zein[302] Although the lives of the animals were prolonged when tryptophan was added to the ration, it remained for Osborne and Mendel[303] to furnish the complete story a few years later by showing that zein plus tryptophan plus lysine promoted normal growth. In this way, tryptophan and lysine were established as essential nutrients

As is the case of all of the amino acids, the function and metabolic interrelations of tryptophan in the organism are still not fully understood Its importance is obvious, since it is a constituent of plasma protein, hemoglobin, thyroglobulin, Nissl substance and many other body proteins The role of pyridoxine in the metabolism of trypotophan is a most interesting one which is discussed elsewhere (page 235).

An even more important aspect of tryptophan metabolism is its relationship to nicotinic acid. The often noted connection of maize with the pellagra syndrome in man, together with the effects produced in experimental animals by diets containing large quantities of cornmeal, naturally focused attention on tryptophan, in which this grain is deficient. Even when nicotinic acid seemed to be the final answer to pellagra and to blacktongue, certain discrepancies could not be explained (page 219). Based on experiments on rats, dogs and swine, which all appeared about the same time, the hypothesis was advanced that tryptophan was a precursor of nicotinic acid. This was shown to be the case in rats and the various intermediates in the transformation of tryptophan to nicotinic acid were worked out by the iso-

tope technique.[326] The following compounds are formed in order from tryptophan: formylkynurenine, kynurenine, hydroxykynurenine, hydroxyanthranilic acid and nicotinic acid. At least three other vitamins affect these reactions. Hence, deficiencies of thiamine, riboflavin and/or pyridoxine may lead to impaired formation of nicotinic acid Thiamine affects the transformation of tryptophan to formylkynurenine. Riboflavin acts on the reaction: kynurenine to hydroxykynurenine; while vitamin B_6 deficiency impairs the reaction hydroxykynurenine to hydroxyanthranilic acid Now that this relationship has been established, pointed studies of uncomplicated tryptophan deficiency, i.e., in the presence of adequate nicotinic acid, are much needed

The experiments of Hopkins[302] in mice and of Osborne and Mendel[303] in rats, established that tryptophan deficiency leads to a disturbance in growth. That this amino acid is also necessary for the maintenance of nitrogen equilibrium in the adult organism has been demonstrated in the rat,[329] mouse,[324] pig[327] and dog.[325]

Loss of hair is a prominent feature of tryptophan deficiency in the rat.[330, 331, 332] Alopecia begins on the head and spreads to involve the face and back.[330] In the growing animal the hair may be restored by adding tryptophan to the deficient diet. No histological studies have been reported thus far.

Cataracts which may not be specific (page 88) have been described in rats[316, 333, 334, 335] and swine[337] In the first species, two types, acute and chronic, have been designated. The former starts in the posterior cortex of the lens, spreads to involve the perinuclear and anterior cortical zones and usually matures within two to three weeks. The latter, which takes longer to appear and matures more slowly, is confined to the anterior and posterior cortices Cataracts developing in tryptophan-deficient rats have been further characterized by their reversability and irreversibility. In the former type there is splitting of the fibers and vacuolization, as a result of excess hydration With disorganization of the lens structure the damage becomes irreversible.[335] "Congenital" cataracts have been noted in rats born to females which had been placed on tryptophan-deficient rations before delivery.[336] Cataracts have been described in growing rats but not in adult animals. In two of three swine studied by Wintrobe *et al.*[337] opacities were noted in the equitorial portion of the lens, in one animal these spread to the anterior and posterior portions of the lens.

Corneal vascularization occurs in rats deprived of tryptophan, in such animals this change appears to be non-specific manifestation of the deficient state.[330] A slight anemia has been reported in growing rats maintained on a diet whose protein was furnished by acid hydrolyzed casein; the blood

count returned to normal when tryptophan was administered.[338] A much more marked reduction in red blood cells and hemoglobin has been observed in swine fed an acid hydrolysate of casein.[339] This anemia is normocytic The reduction in red blood cells is accompanied by normal serum iron levels, no hemosiderosis is found in the tissues. The bone marrow is not particularly hyperplastic If dogs are rendered anemic by bleeding, accelerated hemoglobin formation takes place when tryptophan is administered.[312]

A reduction in plasma proteins has been observed in rats[340] and in swine[337] made deficient in tryptophan In the latter species the serum protein concentration may fall to low levels, for instance, in one animal the normal value of over 6 gm per cent decreased to a low of 2 8 gm. per cent after 117 days on the deficient diet. Tryptophan leads to prompt regeneration of plasma protein in the hypoproteinemic-plasmapheresed dog.[326]

Absence of development of the yellow pigmentation of the incisor teeth has been observed in the rat[330] This, as might be expected, is only seen in growing animals, its pathogenesis is not clear.

Necrosis and atrophy of skeletal muscle have been observed in three swine deficient in tryptophan[337] Such changes resemble those which are so familiar in many species deficient in vitamin E No alterations in skeletal muscle fibers have been noted in rats On the other hand, prominent alterations are found in the myocardium and smooth muscle of the gastrointestinal tract, bladder and uterus in this species Such changes consist of focal necroses with cellular infiltrations[341]

Several observers[340, 341] have noted fatty livers in tryptophan-deficient rats. It has been stated[342] that "tryptophan is a most important dietary essential for normal gestation in the rat" This is true, though the effect is entirely non-specific The differences which have been observed are undoubtedly the result of inanition, since the deficient rats lost, on the average, 34 gm., while the controls gained, on the average, 31 gm In addition, the food consumption of the latter group was greater than that of the former. It seems only logical to assume that reproduction cannot take place when any of the ten essential amino acids are absent, since by definition all are necessary for normal growth

Microscopic examination of the teeth of tryptophan-deficient rats[373] has revealed only non-specific changes

In experimental tryptophan deficiency in the *human*, studies have been carried out for only very short periods. Aside from a negative nitrogen balance, which indicates that this amino acid is indispensable, no significant changes have been observed in infants[343] and adults[344] The direct rela-

tionship of tryptophan to nicotinic acid synthesis in man now seems clear enough since this transformation has been shown by studies utilizing radioisotopes.[345]

LYSINE

Lysine and tryptophan were the first indispensable amino acids to be recognized. From the preceding section it will be recalled that Hopkins[302] showed that the growth of mice could be prolonged when tryptophan was added to zein, and that Osborne and Mendel[303] later showed that growth can be much improved when lysine is added to the tryptophan-zein mixture. The latter investigators also demonstrated that rats fail to grow on a diet of which the protein is supplied by wheat gliadin which is deficient in lysine; growth was improved when the missing amino acid was added.

Lysine is converted via acetate to glutamic acid. It may also be a precursor of heme[347] and a source of arginine.[348] This amino acid has some lipotrophic affect on the fatty livers which occur in the rat on a low protein diet[349]

Lysine is necessary for the growth of rats.[350, 351] Slight though definite decreases in red blood cells and hemoglobin content are seen as a result of lysine deficiency;[352] this may be brought out by studying the effect of lysine administration after a severe hemorrhage.[316] Osseous changes, which are non-specific and consist of diminished endochondral bone formation, are seen in lysine-deficient rats; the healing of artificially produced bony defects in such animals is unimpaired[353] Lysine is a chromotrichia factor in rats[354] The amino acid is an indispensable nutrient for mice,[324] dogs[325] and swine.[327, 355]

Studies of lysine deficiency in the adult human male have demonstrated the development of a negative nitrogen balance[356] The daily need for lysine is set at .8 gm

HISTIDINE

The indispensability of histidine for the rat was demonstrated by Rose and Cox in 1924.[357]

Histidine is a precursor of carbohydrate, since glycogen deposition in the liver follows its ingestion. The ketonuria produced by a high fat diet is decreased when histidine is fed.[358]

Histidine contains the imidazole ring, a structure which the organism

appears to synthesize with difficulty.[359] Hence, it is of interest that the concentrations of carnosine and anserine which contain this ring are decreased in striated muscle of histidine-deficient rats[360]

The relation of histidine to histamine is not entirely clear. Certain tissues contain an enzyme which will form histamine from histidine; for instance, kidney cells have a decarboxylase, which effects this transformation.[361] It is possible that the toxic effects which follow histidine administration may result from its conversion to histamine, all of which may explain the anorexia, tachycardia, respiratory difficulty, and paralysis of the hind extremities which may be observed in rabbits into which the amino acid has been injected Microscopic examination of such animals reveals pulmonary edema and constriction of the bronchial musculature.[362]

Histidine is necessary for growth[357, 363] and for nitrogen balance[364] in the rat. Animals acutely deficient in histidine develop a moderate anemia; hemoglobin values fall from the normal of 14 gm to about 10 gm. per cent Histological examination of the tissues have failed to reveal any specific changes other than those which must be ascribed to inanition or protein deficiency in general.[365, 366] Histidine is needed for the weight maintenance of adult rats[323] Deficiency of this amino acid leads to a negative nitrogen balance in dogs.[325] Histidine is necessary for plasma protein production in the plasmapheresed dog,[326] and promotes hemoglobin formation in dogs made anemic by bleeding. However, the relation of histidine to hemoglobin synthesis in this species requires further study. Histidine is an essential nutrient for swine[367] and mice.[324]

In man, diets deficient in histidine do not lead to a negative nitrogen balance, at least for periods lasting up to eight days.[368]

ARGININE

The indispensability of arginine for the growth of young rats was first shown in Rose's laboratory in 1930[369] This amino acid is not necessary for the well being of adult rats, since sufficient amounts can be synthesized to maintain the organism in nitrogen balance[370]

Arginine administration leads to the deposition of a small amount of glycogen in the liver and to a decrease in the ketosis of fasting rats which have been fed sodium butyrate[371] Arginine is one of the precursors of creatine[372] and, of course, acts as a catalyst in the synthesis of urea

As noted above, arginine is an indispensable nutrient for the growing rat, but is not necessary for the adult rat,[370] mouse,[324] or dog,[325] all of which can synthesize sufficient quantities of the amino acid. *Growing swine need*

tionship of tryptophan to nicotinic acid synthesis in man now seems clear enough since this transformation has been shown by studies utilizing radioisotopes.[345]

LYSINE

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Studies of lysine deficiency in the adult human male have demonstrated the development of a negative nitrogen balance.[356] The daily need for lysine is set at .8 gm.

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Histidine is a precursor of carbohydrate, since glycogen deposition in the liver follows its ingestion The ketonuria produced by a high fat diet is decreased when histidine is fed.[358]

Histidine contains the imidazole ring, a structure which the organism

may be the case is brought out by pigmentary alterations in the hair, which may be returned to normal if tyrosine is administered.[381]

LEUCINE

Leucine was shown to be an indispensable nutrient for the growth of rats by Womack and Rose in 1936.[382]

Leucine labeled with carbon 14 has been shown to give rise to acetate.[383] Little else is known of the metabolism of this amino acid.

No specific histological alterations have been observed in rats which have been placed on leucine-deficient diets.[384] Corneal vascularization and hypophyseal changes would appear to be similar to alterations associated with other amino acid deficiencies.[319, 320] Leucine is of importance in the correction of red blood cell and hemoglobin regeneration after severe hemorrhage.[316] This amino acid is also necessary for plasma protein formation[326] and hemoglobin synthesis[312] in the dog. Leucine is an indispensable nutrient for the suckling pig[385] and the mouse.[324]

In man a negative nitrogen balance ensues when leucine is omitted from the diet. The daily adult requirement is set at 1.1 gm.[386]

ISOLEUCINE

The indispensability of isoleucine for the growth of rats was demonstrated by Womack and Rose in 1936.[382]

Little is known of the role of isoleucine in nutrition save that it is a source of glycogen (pyruvate) and of ketone bodies (acetoacetate).[366]

When rats are placed on an isoleucine-deficient diet growth ceases and weight loss ensues.[382] Non-specific alterations in the hypophysis, gonads, thymus and bone are found at autopsy.[387] The only changes which may be specific is the presence of hyaline and fragmented striated muscle fibers in the only muscle (sternomastoideous) which appears to have been examined. Although the description of this change is inadequate, the illustrations show a definite lesion. It is unfortunate that its extent was not determined. Isoleucine is of significance for hemoglobin and plasma protein production in rats depleted by bleeding.[316]

In the dog, isoleucine is necessary for hemoglobin[312] and plasma protein formation[326] and for the maintenance of nitrogen balance.[325] The pig requires isoleucine,[388] as does the mouse.[324]

Isoleucine is indispensable for infants[389] and for adults[386] since negative nitrogen balance develops in its absence. The daily requirement for the adult male has been set at .7 gm.[386]

a source of dietary arginine.[327] Arginine, therefore, has a unique place between the indispensable and dispensable amino acids. There is some evidence that arginine is necessary for plasma protein formation in the protein-depleted dog[326] and for hemoglobin formation in dogs rendered anemic by bleeding.[312]

PHENYLALANINE

In 1934, an important report from Rose's laboratory showed that phenylalanine is an indispensable amino acid and that tyrosine is not.[374]

The normal metabolism of phenylalanine calls first for its conversion to tyrosine, further breakdown is indicated by an opening of the benzine ring[375] Phenylalanine and tyrosine may be metabolized to form acetoacetate, which is of significance in lipid turnover.[426] Transamination may lead to keto acids which may then undergo decarboxylization. The role of ascorbic acid in phenylalanine metabolism, which has been shown in both guinea pigs and premature babies, is discussed on page 176. Phenylalanine and tyrosine are structurally very closely related to epinephrine, ephedrine, thyroxin, triodothyronine, and melanin, although no changes in the metabolism of such compounds have been reported in animals deficient in these amino acids.

When rats are placed on diets deficient in phenylalanine, growth promptly ceases,[374, 376, 377] a negative nitrogen balance also ensues.[323] Pathological studies have been reported in two series of phenylalanine-deficient rats and their paired-fed controls.[376, 377] An average reduction in hemoglobin from 14.7 gm. to 9.9 gm. and a slight fall in plasma protein concentration from 5 58 gm. per cent to 4.71 gm. per cent are described[376] Histological examination of the tissues has shown no changes other than those which can be ascribed to inanition: disturbance in endochondral bone formation, thymic atrophy, decrease in size and in fat content of the adrenals, and atrophy of the testicular tubules.

In dogs phenylalanine appears to be necessary for plasma protein production[326] and for the maintenance of nitrogen balance[325] Phenylalanine is an indispensable amino acid for swine[327] and mice.[324]

When normal infants are placed on a phenylalanine-deficient diet, they cease gaining weight and evidence impaired nitrogen retention[378] This amino acid is indispensable for the adult human male in whom the daily requirement is placed at 1.1 gm.[379] Such a requirement for phenylalanine may be lowered if tyrosine is fed since the latter amino acid may spare 50 to 75 per cent of the needs of the former.[380]

The primary disturbance in the syndrome of phenylketonuria is a block in the conversion of phenylalanine to tyrosine. Hence, the disease may be looked upon, in part, as a form of conditioned tyrosine deficiency. That such

cystine, were considered to be indispensable components of the diet. At that time, however, Rose and his co-workers[400] demonstrated cystine to be a dispensable nutrient, for when adequate amounts of methionine were added to a cystine-free amino acid mixture, growth of rats fed a ration with such a source of nitrogen was normal That methionine may be converted into cystine by the rat further clarified the relationship of these two amino acids

The metabolism of methionine is thus closely related to that of cystine, and in turn to choline, creatine and substances which include a host of sulfur-containing compounds, such as those enumerated on page 251. Methionine is indispensable except under stringent laboratory conditions, i e., when homocystine and choline are fed to rats on a methionine-free diet, the latter amino acid will be formed[957]

Methionine donates methyl groups to ethanolamine, resulting in the formation of choline[958] (page 251) The stages in the transformation of methionine to cystine are thought to be as follows: methionine→homocysteine + methyl groups; homocysteine + serine→cystathione→cysteine →cystine The sulfur of the cystine which results is derived from methionine, the carbon chain from serine

Since the metabolism of methionine and choline are so closely related, it has been difficult to separate the pathological manifestations of a deficiency of one from the other (page 251). So, too, since methionine is a precursor of cystine, it has been difficult to separate entirely the effects of deficiency of either of these two amino acids. For when methionine is absent from the diet, cystine obviously will be too, unless otherwise supplied.

An antagonist of methionine, ethionine, which contains an ethyl radicle in place of the methyl portion of methionine, has been used to study certain effects of methionine deficiency.[401]

Methionine is necessary for the maintenance of nitrogen metabolism in the adult rat[323] and the dog.[325] In addition, it is apparently necessary for growth of the mouse[324] and for hemoglobin formation in anemic dogs[312]

When methionine deficiency is produced in the growing rat by utilizing a mixture of purified amino acids, including cystine, there is retardation of growth[402] Moreover, a reduction in hemoglobin formation and in plasma protein production is seen as well No alterations are found in the liver and other tissues at autopsy. That methionine plays a role, though how specific a one, in protein synthesis is brought out by further studies on rats[402] and dogs,[403] in both species reduction in hemoglobin content, red blood cell count and plasma protein concentration has been reported.

From the data which have been accumulating during the past 20 years or so, it is now clear enough that the effects of methionine and cystine deficiencies, particularly the latter, are different from those of choline (page

THREONINE

While studying the problem of essential and non-essential amino acids, Rose and his co-workers[390] discovered a new one which they added to the indispensable group in 1936. After proving that it had an analagous structure to d-(-)-threose, the new amino acid was named threonine.[391]

The metabolic role of threonine has been partially elucidated in recent years as a result of isotopic studies.[392] This amino acid appears to be a precursor of glycine and of acetate. Thus, as might be expected, threonine could be a source of heme. Unlike most amino acids, threonine does not appear to participate in transamination reactions.

The effects of threonine deficiency have been studied in the rat.[393] On a diet containing all crystalline amino acids save threonine, no disturbances other than those which could be ascribed to general inanition appear to have been observed. The usual non-specific effects on bone, thymus, hypophysis and gonads were found when these and other tissues were examined microscopically.

Studies of the influence of threonine on the deposition of fat in the livers of rats fed diets of 9 per cent casein indicate some lipotrophic activity by this amino acid.[394] Moreover, diffuse fatty infiltration has been noted in the livers of rats fed diets completely deficient in threonine.[395] The fatty infiltration, which may be corrected by threonine, is less severe than that which is observed on low-protein diets, which are deficient in choline[396, 397] Threonine is an essential nutrient for mice,[324] dogs[325] and swine.[327, 398] It is necessary for hemoglobin formation[312] and plasma protein production[326] in dogs. Mice exhibit a rapid loss of weight and exhibit a peculiar puffy appearance and marked abdominal distention.[324] At autopsy severe edema and ascites are found.

In infants threonine has been shown to be indispensable.[399] The requirement of 60 mg. per kg. per day has been set. Below this, there is failure to gain weight in normal fashion and impaired nitrogen retention.

Threonine is also an essential nutrient for the adult human. The requirement has been set at .3 to .5 gm. per day.[368] It is of interest that in Wilson's disease, in which amino aciduria is prominent, threonine is excreted in amounts larger than those of other amino acids.[297]

METHIONINE

Until 1937 both of the sulfur-containing amino acids, methionine and

ency was interesting many workers in the production of fatty livers and cirrhosis. The situation was therefore somewhat confused when György and Goldblatt[illegible] described fatty infiltration, necrosis and cirrhosis of the livers of rats which had subsisted on a diet low in the B vitamin group, though adequate in protein content. In 1942, however, Daft, Sebrell and Lillie[illegible] clarified the situation by separating what appeared to be the primary effect of cystine deficiency, that is, hemorrhagic necrosis of the liver, from those effects which could be ascribed to choline deficiency, which are fatty infiltration and cirrhosis.

Experimental procedures employing crystalline amino acid mixtures supplemented with methionine and/or cystine in the presence of adequate choline were then reported[illegible] and confirmed the conclusions just cited.

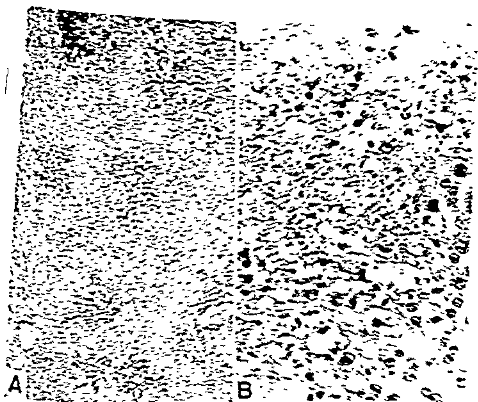


Figure 43. Cystine Deficiency

Liver, rat. Section from animal on low protein, high fat diet with tocopherol but no added cystine. A (×85), B (×480). Note diffuse necrosis with patchy foci of liver cells remaining which bear no relation to the lobule. Higher power (B) shows transition between necrotic areas and remaining liver cells. Note red blood cells, pyknotic nuclei and a few leukocytes. H. and E.

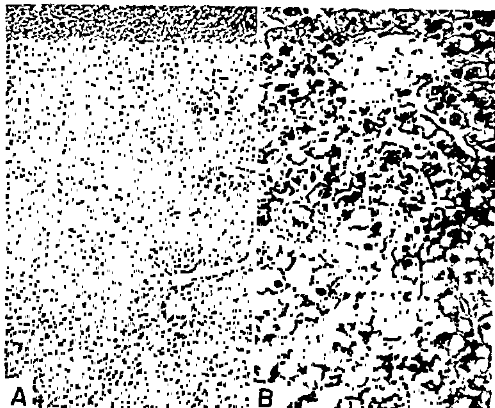


FIGURE 42. CYSTINE DEFICIENCY

Liver, rat. Sections from animal which had been on low protein, high fat diet with tocopherol but no added cystine *A* (x 75); *B* (x 450). Extensive necrosis is present which tends in this animal to involve all tissue but the cells about the central veins All necrotic cells appear to have been involved at about the same time. In the higher power *(B)* note sharp transition for viable appearing cells to those which are completely necrotic. No leukocytes or red blood cells are present H and E.

251. It would appear that this is the most appropriate place to discuss the syndrome of massive hepatic necrosis and the role played by cystine and other factors in its pathogenesis Although a great deal has been learned during the past 10 years, the story is not yet complete.

Investigations of cystine deficiency began in 1935 with the studies of Weichselbaum [404] who noted jaundice before death and at autopsy gross hemorrhages in the livers of rats fed a diet low in protein and cystine content. When cystine was administered in the basal diet to such animals, the hemorrhagic changes and deaths failed to occur. Moreover, moribund rats could be saved by the administration of cystine, but not methionine These experiments were carried on just at the time when studies of choline defi-

ciency were interesting many workers in the production of fatty livers and cirrhosis. The situation was therefore somewhat confused when Gyorgy and Goldblatt [405] described fatty infiltration, necrosis and cirrhosis of the livers of rats which had subsisted on a diet low in the B vitamin group, though adequate in protein content. In 1942, however, Daft, Sebrell and Lillie [406] clarified the situation by separating what appeared to be the primary effect of cystine deficiency, that is, hemorrhagic necrosis of the liver, from those effects which could be ascribed to choline deficiency, which are fatty infiltration and cirrhosis

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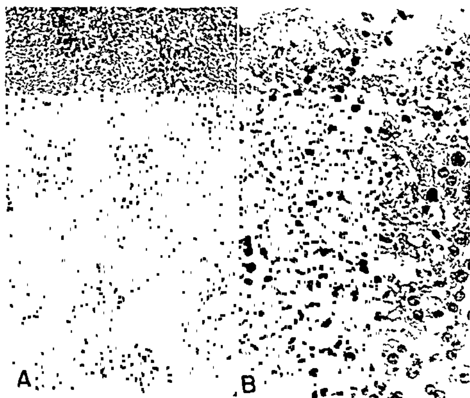


FIGURE 43 CYSTINE DEFICIENCY

Liver, rat Section from animal on low protein, high fat diet with tocopherol but no added cystine A (x 65), B (x 450). Note diffuse necrosis with patchy foci of liver cells remaining which bear no relation to the lobule Higher power (B) shows transition between necrotic areas and remaining liver cells. Note red blood cells, pyknotic nuclei and a few leukocytes H. and E.



FIGURE 44. CYSTINE DEFICIENCY.

When groups of rats were placed on diets whose protein content was supplied by mixtures of crystalline amino acids containing varying amounts of methionine and cystine, the following differences were noted. When both methionine and cystine were absent, the animals lost weight and developed an uncharacterized anemia and hypoproteinemia; at autopsy, extensive hemorrhagic necrosis of the liver was found. When inadequate amounts of methionine but no cystine were added to the diet, the animals lived a little longer but succumbed with liver necrosis, even though no anemia or hypoproteinemia had developed. When methionine intake was restricted but dietary cystine was adequate, no liver necrosis was observed although during life disturbance in hemoglobin and plasma protein formation developed. Thus, methionine deficiency appeared to interfere with hemoglobin and plasma protein formation, while a lack of cystine seemed to lead to liver necrosis which developed in the absence of fatty infiltration, since choline was present in the diet. Hence, by the mid 1940's, there was general agreement that cystine was an important factor for the liver, since a deficiency of

this amino acid was responsible for the development of massive hepatic necrosis in the rat.

However, results obtained in laboratories where further studies of hepatic necrosis were being carried out indicated that cystine deficiency was not the entire answer. Another factor, alpha-tocopherol, proved to be of importance, since it could protect rats which had been placed on diets which ordinarily led to massive necrosis of the liver.[676] This observation has been confirmed in many laboratories; moreover, the deleterious effects of cod liver oil and other unsaturated lipids which destroy vitamin E has been demonstrated.

But the story was far from completed. Certain diets high in yeast content were found to be necrogenic.[1478] This activity could be abolished by a substance found in casein, now designated as Factor 3,[699] whose activity may be related to its selenium content.[213]

At the present writing it is clear that the massive hemorrhagic necrosis produced by dietary means is not the result of a single deficiency. At least three factors: cystine, vitamin E, and Factor 3, which may be selenium, appear to be implicated This brings up the whole question of differentiating a deficiency state from an intoxication, since many poisons are well-known causes of hepatic necrosis. Whether any one or all of the three aforementioned factors play a role in detoxification mechanisms is a question which remains to be elucidated.

Structural, as well as biochemical, alterations which are found in the course of the massive hepatic necrosis syndrome of rats have been described[407, 408] During the prenecrotic stage the liver cell exhibits a reduction of cytoplasm and a decrease in the number of basophilic ribosenucleic acid granules Such alterations are similar to those which develop in the liver as a result of uncomplicated protein deficiency Although an increase in stainable lipid may be encountered, necrosis will develop in its absence. Death of the liver cells is sudden and widespread Ordinarily, demise of the animal occurs within twenty-four hours. The few rats which recover develop irregular depressed scars in the liver

A serial analysis of certain constituents in the liver during the development of the massive necrosis has revealed that in the prenecrotic stage a fall in wet and dry weight of the liver occurred. The concentrations of total lipids, neutral fat, total fatty acids and cholesterol esters progressively increased Glycogen and phospholipid content remained unchanged. With the development of necrosis a significant increase in wet and dry weight was seen. In addition, an increase in free cholesterol and a decrease in glycogen and phospholipid concentrations occurred

The picture of massive liver necrosis may be altered in several ways For instance, the administration of dessicated thyroid gland leads to the

development of more extensive lesions[409] Thiouracil and certain other "anti-thyroid" drugs tend to protect. Pregnancy appears to be a precipitating factor in the development of experimental dietary necrosis in the rat.[410] When the carbohydrate content of the ration is varied, no discernible effect can be demonstrated on its necrogenicity.[411] On the other hand, raising the fat content of the diet appears to increase the degree of liver cell damage.

The relationship of methionine to the maintenance of the integrity of the liver of protein-depleted dogs in the presence of poisons is another interesting story. Any results, of course, may be due to a choline or cystine effect When dogs are placed on a low protein intake and their protein stores are reduced by plasmapheresis, a reduction in tolerance to the intravenous administration of mapharsen occurs as measured by the appearance of jaundice[412] The administration of methionine the day before the injection of the arsenical raises the tolerance of the animal in that larger doses of the drug are required to produce icterus. Then, too, if similar protein-depleted dogs are maintained under chloroform anesthesia for thirty minutes, they die of necrosis of the liver.[413] Methionine has a dramatic effect on such dogs, if within three or four hours following the anesthesia this amino acid is injected intravenously, the animals recover. It is unlikely that such protective effects can be observed in normal dogs.[414]

Since methionine is a precursor of cystine, what little else that is known of the role of the latter amino acid in the organism should be mentioned Cystine has been known to be a constituent of hair for some time, when various specimens of hair are analyzed, they are found to contain on the average almost 20 per cent of this amino acid[415] That rats placed on a cystine-deficient diet will show an inhibition of hair growth is therefore not an unexpected finding[416] The hair of such animals is also abnormal in that the medullary cells are broader and more loosely packed; the cortical portion, which incidentally contains sulfur, is narrower than control hair. It is unfortunate that histological studies of the skin of such animals have not been made. Other experiments employing diets deficient in sulfur containing amino acids indicate a slightly increased production of hair when methionine is added into the diet.[417]

Some incidental observations of methionine deficiency in rats and other species might be mentioned.

When the corneas of methionine-deficient rats are examined with the slit-lamp, vascular buds are found growing towards the center of the structure.[418] This would appear to be a nonspecific effect (page 453).

Methionine is an indispensable nutrient for swine[419] The methionine requirement for the maintenance of nitrogen metabolism in adult man has been set at 1.1 gm. per day.[420] As might have been anticipated from the studies reported in experimental animals, cystine exerts a sparing effect

on methionine, in fact the former amino acid can replace 80 per cent of the requirement of its precursor.[421] This is far more than the 30 per cent replacement of cystine for methionine in the rat

VALINE

The last of the amino acids shown to be indispensable for the rat was valine; Rose and Eppstein reported this in 1939[422]

Valine is a source of carbohydrate (glycogen)[423] Other roles of this amino acid in metabolism are unknown at this time

When rats are placed on a valine-deficient diet, they virtually stop eating and rapidly lose weight. In the terminal stages of the deficiency they exhibit unique signs which Rose and Eppstein[422] described as follows· "The rats become extremely sensitive to touch and display a severe lack of coordination in movement. They walk with a staggering gait As the animal attempts to walk the left foreleg is raised inordinately and the head is retracted Frequently the subjects show a rotary motion resembling that of a dog chasing its tail This may be either clock or counter-clockwise and may continue until the animal falls to the floor of the cage from sheer exhaustion As would be anticipated the symyptoms are rapidly cured by the administration of valine without any other therapeutic measure" No histological studies in such animals have been reported and, of course, are greatly to be desired Valine is needed for hemoglobin and red blood cell regeneration in the rat made anemic by hemorrhage[316]

Valine is necessary for growth of mice[3-4] and for plasma protein and hemoglobin formation in the dog[312, 120] Growth and feed utilization is impaired in swine which have been placed on a valine-deficient ration.[424]

In man valine is well-recognized as an indispensable nutrient. A level of 8 gm per day in the diet is necessary to insure positive nitrogen balance.[425]

MISCELLANEOUS AMINO ACIDS

GLYCINE

The simplest amino acid, glycine, is of great importance metabolically since it is a precursor of a great many nitrogenous and non-nitrogenous compounds.[426] These include serine (and hence ethanolamine and choline, page 251), glutathione, creatine; a variety of proteins, including collagen which contains 25 per cent glycine, prophyrins, uric acid, purines, formate, pyruvate and acetate. The relation of folacin and vitamin B_{12} to the metabolism of glycine is discussed on pages 271 and 277.

Since all mammalian organisms can synthesize glycine, this amino acid belongs in the dispensable group. However, the glycine in the organism may be depleted if it can be diverted away in sufficient amounts. In the experimental animal this may be accomplished by feeding benzoic acid, which combines with glycine to form hippuric acid. When such an experimental procedure is applied to growing rats, weight gain is retarded.[428]

SERINE

Serine may be formed from glycine[427] and is the precursor of ethanolamine which is then transformed into choline.[950] Serine also participates in the formation of cystine, is a source of pyruvate, and may give rise to alanine[429]

PROLINE AND HYDROXYPROLINE

These two amino acids are of importance because of their high concentrations in the collagen molecule.[430] Together they furnish almost one quarter of the amino acid residues found in collagen from various mammalian sources. The presence of hydroxyproline in collagen is so unique that its concentration in specimens is used as an index of their collagen content.[431] One source of proline would appear to be ornithine.[432]

GLUTAMIC ACID

This amino acid deserves mention because of its probable role in the metabolism of nervous tissue.[433]

UTILIZATION OF D-AMINO ACIDS

Study of the D forms of the amino acids indicates that some of these may be utilized for growth, of rats at least. Among the indispensable group these include: histidine, methionine, phenylalanine, tryptophan, and valine.[434]

Perhaps it was somewhat naive to think, as we must confess doing a dozen years ago, that, when deficiencies of single amino acids were produced, many interesting lesions would turn up This train of thought was contrary to the all or none principle of amino acids in protein synthesis. As must be obvious from the monotonous description of the alterations described in the preceding pages, deficiencies of single amino acids produce changes similar to those following protein deprivation. These include defects in plasma protein and hemoglobin formation and non-specific alterations in a number of tissues: cartilage and bone, thymus, gonads, hypophysis, cornea, and lens. Hence, when one totals up the specific changes which may be ascribed to single amino acid deficiencies, the list is not an

imposing one, as Table V will indicate. This does not mean, however, that one should cease such studies, particularly with respect to biochemical lesions. Moreover, studies of partially-deficient states might be more rewarding than those produced by total deprivation which have been investigated so far.

TABLE V

A SUMMARY OF SPECIFIC ALTERATIONS ASSOCIATED WITH DEFICIENCY OF SINGLE AMINO ACIDS

(1) *Tryptophan*
- (a) Niacin deficiency
- (b) Alopecia, rat
- (c) Loss of pigment, rat's incisor
- (d) Necrosis, muscle skeletal (swine), cardiac and smooth (rat)
- (e) Fatty liver

(2) *Lysine*
- (a) Hair pigment, rat
- (b) Fatty liver

(3) *Isoleucine*
- (a) Necrosis, skeletal muscle, rat

(4) *Threonine*
- (a) Fatty liver

(5) *Methionine (Cystine)*
- (a) Choline deficiency
- (b) Growth of hair
- (c) Necrosis of liver

(6) *Valine*
- (a) Convulsions

In the Preface and elsewhere in this monograph the importance of the association endogenous factors with deficiency disease has been mentioned. In this respect protein deficiency looms large This is because many of the conditioning or endogenous factors, which are associated with deficiency states, are genetically mediated. If one accepts the one gene–one enzyme hypothesis and since enzymes are proteins, it is not too unreasonable to develop the concept that genetically controlled diseases can be looked upon as manifestations of protein deficiency It may come as a shock to some to realize if such a view is carried to its logical conclusion, that, for example, scurvy in the guinea pig, primates, and man must be viewed basically as a manifestation of protein deficiency since such species lack the protein enzyme which is necessary to synthesize ascorbic acid It is not necessary to carry this discussion further at the present time Some day the subject of deficiency disease may have to be reviewed and rewritten from this standpoint.

Part IV

Lipids

PART IV

LIPIDS

LIPID METABOLISM IN GENERAL

Fat is of importance in the diet for several reasons. First off, lipids have the energy value of nine calories per gram, which is higher than the four calories yielded by protein or carbohydrate Next, fat, at least as far as the human diet is concerned, improves the taste of food The importance of the essential fatty acids will be discussed below. Lastly, the fat-soluble vitamins, which are discussed on pages 125 to 173, occur in many of the natural fats.

Except for the specific essential fatty acids, the remainder of the various types of lipids are considered dispensable Body fat can, of course, be formed from carbohydrate and protein However, it appears that the inclusion of fat in the diet of rats improves their growth and reproductive performance[435] Moreover, the type and amount of such dietary fat is important.[436] The presence of an excessive amount of fat in the diet, particularly that of man, has attracted a good deal of attention recently, particularly with respect to the possible role of lipid ingestion on the development of arteriosclerosis[437]

Ingested fats are broken down in the intestinal tract into fatty acids and glycerol.[438] The presence of intestinal dysfunction (diarrhea, sprue syndrome), the absence of certain secretions (bile, pancreatic juice), or anatomic alterations of the intestinal mucosa, all may interfere with absorption. This is particularly important with respect to the fat soluble vitamins and to the essential fatty acids Lipids of high melting points are poorly absorbed.

After the fatty acids are absorbed they go to the liver Recent studies have demonstrated the pathway of their metabolism here, where coenzyme A plays the dominant role[439] It will be recalled that pantothenic acid is a part of coenzyme A (CoA)[888] The long chain fatty acids are activated by CoA to form the acyl derivative, which is then oxidized at the beta carbon. This reacts to form acetyl-CoA and acyl-CoA with the loss of two carbon atoms, a process which continues until the fatty acid is completely oxidized The acetyl-CoA, as might be expected, enters the Krebs cycle (page 223) Further aspects of lipid metabolism, particularly with respect to choline, are discussed on page 251

THE ESSENTIAL FATTY ACIDS

In 1929, Burr and Burr[440] described a new syndrome in rats which had been placed on a diet devoid of fat. This disease was characterized by growth failure, which appeared after four or five months. During life, changes in the skin were prominent; while at autopsy kidney lesions were observed The manifestations of this disease could be cured by as little as ten drops of lard each day Burr and Burr[441] soon showed that the specific factor in lard was the doubly unsaturated fatty acid linoleic acid; this was therefore designated as an "essential fatty acid." Linolenic and arachidonic acids, with three and four unsaturated bonds, respectively, have since been shown to substitute for linoleic acid.[443]

Of these three fatty acids linoleic appears to be the primary one, hence it must be supplied in the diet.[443] It may give rise to arachidonic and linolenic acids The reverse is not possible. The unnatural isomers of linoleic acid have virtually no activity The best sources of the unsaturated fatty acids are vegetable oils (corn, cottonseed, peanut, and soybean). Hydrogenation of these oils reduces the content of naturally occurring essential fatty acids.

The biochemical role of the essential fatty acids is obscure. Radioisotope studies have made it clear that these compounds cannot be formed by the organism[442] Unsaturated fatty acids appear to be related to the deposition of ceroid (page 258) In the rat and mouse, fatty acid deficiency is accompanied by an increased consumption of water; the average daily intake of deficient and control rats has been reported as 20 9 and 1.5 cc., respectively[441] The total oxygen consumption of deficient animals is increased[443]

Clear-cut effects of experimental fatty acid deficiency have been reported in growing rats,[440, 444 445] mice[447] and dogs[449, 450, 451] Alterations in adult animals, though harder to produce, have been described in rats[456] and mice[448]

When young rats are placed on a fat-free diet, the initial change is seen after seventy to eighty days, this consists of scaling of the epidermis over the dorsa of the feet Scaling of the tail occurs; the tip of this structure usually becomes inflamed and necrotic. Alopecia of the head, neck and back may also be observed Changes appear more readily in male than in female animals[445]

Microscopic examination[444, 445] of the skin reveals extreme thickening of the epithelial layer, the epidermis increasing from two cells up to six to twelve cells in depth The strateum corneum is markedly hyperkeratotic

and sometimes exceeds the normal epidermis in thickness. The cells of the stratum granulosum have such an abundant mass of cytoplasm that they appear much larger than normal The collagen fibers of the corium appear edematous, more cellular infiltration is found here than in the controls. The epithelial cells of the hair follicles are increased in size, the hairs themselves easily break off Increased capillary fragility has also been observed.[446]

Gross changes have been described in the skin of young mice but not in adult animals.[447] Differences in development of skin changes have been found to vary with the strain of the mouse under study[448] In dogs[449, 450, 451] alterations similar to those seen in the rat have been reported. Grossly, the most conspicuous changes are dryness of the skin, desquamation, alopecia and an increased susceptibility to infection. Studies in this species may have to be carried out for several years since the changes take some time to develop. For instance, dryness of the skin may appear in three months, on the other hand, alopecia may not become apparent for one to two years Histological study reveals thickening of the epidermis and an increase in the cells of the hair follicles. The stratum corneum shows excessive hyperkeratosis, the cells of the structure granulosum are increased in number. In the dermis the collagen fibers are swollen and the tissue is infiltrated with cells The sebaceous glands are enlarged, the hair follicles are plugged with hyperkeratotic material.

The Burrs[440] described "bloody urine" and kidney lesions in fatty acid-deficient rats, they felt that such changes were made more severe by a high protein diet. Grossly, the kidneys are enlarged and pale with finely pitted or coarsely granular surfaces[464] There has been some divergence of opinion when the kidneys are examined microscopically One group[464] has described tubular epithelial cells filled with lipid, some cells are necrotic The tubular lumens contain hyaline material and fat droplets. Calcification is prominent following tubular damage, glomeruli are said to be normal In contrast, recent studies, using more suitable diets, have indicated enlargement of the kidneys but no changes in the nephrons, merely hyperemia.[455] The reason for the discrepancy in these two reports is not entirely clear, though in the earlier study the protein and choline contents of the diet are not specified, these are important in view of renal lesions which have been described in choline-deficient rats (page 259) Kidney changes have not been described in dogs deficient in fatty acids.

Disturbances in reproductive activity have been observed Histological studies have been reported in male and female rats.[452, 453, 454, 455] *In none* has the restricted weight gain technique been used, so that it may be possible that the changes described are due only to *nonspecific interference* with growth. The male animal exhibits a loss of sex interest together with

macroscopic atrophy of the testes. Microscopically, the tubular epithelium shows various degrees of degeneration and giant cell formation. Regeneration takes place following administration of one of the essential fatty acids.

In the female animal, though reproduction is affected early, ovulation goes on until late in the course of the deficiency. There are atrophic changes and under-development of the uterine mucosa; the maternal decidua fails to develop normally. The embryos are either resorbed or remain in utero longer than normal. Hemorrhage and necrosis accompanied by secondary inflammatory phenomena have been observed in the placentae and uterine walls The changes which have been described are reminiscent of those which have been reported in vitamin A deficiency in rats.

Cell counts of the pituitary glands of female [454] and male [455] rats appear to indicate a significant decrease in the acidophiles. It is suggested that fatty acid deficiency may lead to a decrease in LH production since this hormone is thought to be produced by acidophiles. Moreover, changes in the ovary such as the presence of "wheel cells" have been correlated with a deficiency of LH production. Similar decrease in acidophilic cells are seen in male animals.

The fat content of the liver is elevated in fat-deficient animals. Lipid is found in greatest amount about the central vein.[454, 455] More is seen in males than in females.

As noted above, young mice display skin changes in fatty acid deficiency.[447] Adult mice do not, yet such animals are definitely not normal, for when the tips of their tails are cut to remove blood, these structures may go on to develop necrosis or the animal may die. So, too, adult mice are less resistant to x-radiation; reproduction is also impaired.[447] Manifestations of fatty acid deficiency may be produced in adult rats if the intake of the deficient diet is restricted for some weeks; upon feeding the animals *ad libitum*, the skin and other lesions appear.[456] Calves are susceptible to fatty acid deficiency.[457] Retardation of growth, scaly dandruff, dryness of the hair, alopecia, and diarrhea have all been described The effects of low fat diets have been studied in swine.[458]

A group of children with eczema and other skin diseases has been studied by Hansen.[459] In the children with eczema a significant lowering of the iodine number of the fatty acids of the serum was found. With the administration of oils having high iodine numbers the serum values raise with a coincident improvement in the skin lesions. One adult man, who was placed on a fat free diet for six months, showed no clinical changes although the iodine number and linoleic acid content of the plasma fatty acids decreased.[460]

The multiple effects of fatty acid deficiency: on growth, the skin, re-

production, the kidneys and liver, make it difficult to ascribe all changes to a single biochemical defect. The best evidence concerning the function of these unsaturated fatty acids is that they are most intimately concerned with lipid metabolism Such a hypothesis is based on the following: (1) The requirement for the essential fatty acids is increased when the diet contains abundant or excessive amounts of lipid (saturated or unsaturated).[461] (2) Essential fatty acids affect the serum cholesterol level Cholesterol feeding may accelerate the development of fatty acid deficiency in rats[462] So, too, in the presence of hypercholesterolemia, administration of essential fatty acids reduces the concentration of cholesterol in the serum. (3) The effect of fatty acid deficiency on fat deposition in the liver has already been mentioned Moreover, certain changes in the disturbance of unsaturated fatty acids in the subcellular particles of the liver of the rat have been noted in animals deprived of essential fatty acids[463]

Part V
Carbohydrates

In 1920, Osborne and Mendel[466] contributed an article entitled, "Does growth require preformed carbohydrate in the diet?" Their ration, which was fed to rats, consisted of a source of protein (casein, edestin or lean beef extract), inorganic salts, agar-agar, lard and yeast. The answer to the question posed by the title of their paper was, "no" Some years later this same question was asked anew when a diet consisting of casein, crisco, salts, butter fat, and crystalline vitamins was fed, again the answer was in the negative.[467] The outcome of such experiments as these, of course, is to be expected, since two sources of carbohydrate, i e., protein and fat, are present in the rations.

In an ordinary diet carbohydrate may furnish 50 per cent of the calories In certain areas where cereal grains, such as corn and rice, are consumed in large amounts carbohydrate may contribute even more calories to the diet Carbohydrates are used for the synthesis of certain important cellular constituents, such as nucleic acids, galactolipids of the brain, mucopolysaccharides, glycogen, enzymes, et cetera However, the amount of carbohydrate in the organism is not large For instance, it has been estimated that the total carbohydrate in a man weighing 70 kg is only 370 gm, of which 245 and 108 gms are found in muscle and liver respectively as glycogen, and 18 gms are present as the glucose of serum and extracellular fluids.

As already noted, glucose may be derived from protein Certain amino acids are glucogenic. It is estimated that 58 per cent of total ingested protein may be transformed into glucose

Certain organs depend exclusively on carbohydrate for their energy requirements Nervous tissues is the example, *par excellence*, of this phenomenon Heart muscle can metabolize a certain proportion of glucose (page 468) So, too, may skeletal muscle

If one does not encounter carbohydrate deficiency in terms of a decrease in serum glucose level as a result of dietary restriction, may he observe conditioned carbohydrate or glucose deficiency at a cellular level? The answer to this question is, of course, in the affirmative Hence it is necessary to discuss briefly glucose deficiency disease, *i e.*, the hypoglycemic syndrome.

Experimental hypoglycemia may be produced in any of a variety of ways which interfere with the normal transformation of glucose and other sugars to glycogen and the release of the latter from the liver. All these mechanisms are controlled by enzymes which in turn are acted upon by certain hormones. Hence the two principal types of experimental hypoglycemia

are those produced by damage to the liver or by hormonal imbalance, particularly insulin.

It has been recognized for many years that removal of the liver is followed by hypoglycemia during the premortem course of the animal.[468] This is to be expected since the glycogenolytic mechanism in the liver is responsible for maintaining the blood sugar. Less drastic measures which are designed to produce hepatic damage may also lead to hypoglycemia. Such include, interference with the circulation and the adminstration of certain substances: elementary phosphorus, carbon tetrachloride, chloroform and other well-known hepatotoxic agents. Abnormal states associated with fatty infiltration of the liver resulting from choline deficiency (page 251) or extensive necrosis dependent on a deficiency of cystine, tocopherol, or Factor 3 (page 101) may also be accompanied, in severe instances at least, by hypoglycemia. In all of the above disturbances the hypoglycemia and its symptoms are not present alone; other metabolic defects associated with liver disease may even mask the effects of lowered serum glucose.

As might be expected, the most dramatic form of experimental hypoglycemia is produced in the normal organism by the administration of insulin.[1281] Here the hypoglycemic syndrome exists in as pure a form as one may ever see it, for instance, hunger, sweating, nervousness and tremulousness, confusion, convulsions, coma, and death. All these symptoms are probably related to the fall in blood sugar since they are seen after complete removal of the liver.

With the advent of longer acting insulins more prolonged episodes of hypoglycemia may be produced. Such may lead to anatomical alterations in the central nervous system A number of studies have been reported.[298, 299] The most prominent alterations found at autopsy are foci of hemorrhage in the cerebral white matter. Such may be related to the preceding convulsive seizures. Even more impressive is evidence of widespread damage to neurons. The usual chromatolytic changes which end in necrosis are found Certain areas of the brain are affected more than others Such a distribution is similar to that encountered from various causes of hypoxia.

Part VI
The Vitamins

PART VI

THE VITAMINS

INTRODUCTION

The term, *vitamine*, was coined in 1912 by Casmir Funk, who grouped certain diseases· beriberi, scurvy, pellagra, sprue and rickets, and indicated that all, with the exception of pellagra, "can be prevented and cured by the addition of certain preventive substances; the deficient substances, which are of the nature of organic bases, we will call *vitamines*"[469]

Some years later the term was changed to vitamin. Today a vitamin is usually defined as an organic substance, soluble in fat or water, which is ordinarily needed only in minute quantities to maintain the biochemical and structural integrity of many cells and tissues In some instances, we know more of the biochemical than the structural roles, in others, the opposite is true. A number of vitamins are, as we shall shortly see, integral parts of certain organic molecules which are called coenzymes

The story of the discovery of each of the "accessory food factors," or vitamins, begins with the human disease with which the vitamin later came to be identified. In general, a definite sequence of events occurred. (1) Description of the clinical syndrome with some knowledge of its therapy, (2) Production of the disease in suitable experimental animals, and (3) Isolation, chemical characterization and synthesis of an active compound. Such is the story of scurvy and ascorbic acid, of beriberi and thiamine, of xerophthalmia and vitamin A, of rickets and vitamin D, of pellagra and tryptophan-niacin and, in part, of pernicious anemia and vitamin B_{12}. In the transfer of knowledge from each of the human diseases to the syndromes in the experimental animal much new and useful information has been obtained. We can only briefly enumerate some of the important dates with references in the evolution of our knowledge of the vitamins, these are summarized chronologically in Table VI (next page).

With the discovery, chemical characterization, and synthesis of many of the vitamins during the 1930s and with the introduction of radioactive isotopes into biochemistry at the end of that decade, knowledge of intermediary metabolism has advanced greatly. Now a fairly comprehensive picture of the metabolic interrelations of the materials discussed in this monograph, that is, the essential elements, amino acids, fatty acids and vitamins, has been obtained With this in mind, it was felt that a simplified version of some ways in which these materials interact in the metabolism of protein, lipid and carbohydrate might be useful, especially for those who may not have kept abreast of the many recent advances in the field. Figure

TABLE

THE DISCOVERY

NATURAL DISEASE IN MAN	EXPERIMENTAL DISEASE AND ITS PREVENTION
SCURVY - Lind, 1753 (700)	Scurvy, guinea pig, 1907 (702)
BERIBERI - Takaki, 1880's (1341)	"Polyneuritis", fowl 1890's (778) Biochemical lesion, 1936 (757)
XEROPHTHALMIA - Mori, 1904 (1272)	Xerophthalmia, rat, 1913 (1481, 1482) (1)
RICKETS - Park and Howland (1297)	Rickets, dog 1918 (528) rat 1922 (533)
PELLAGRA - Goldberger, 1914-29 (1184, 1185, 1186)	Blacktongue, dog, 1922-28 (1200, 1201) Acrodynia, rat, 1926 (904)(3) Skin lesions, rat, 1938 (905) Growth, rat, 1939 (866) Egg white injury, 1927 (1012) Alopecia, mouse, 1940 (1033) Achromotrichia, rat, 1941 (1040)
DIABETES MELLITUS - Banting and Best, 1922	Fatty liver, dog, 1922-30 (953)
PERNICIOUS ANEMIA - Minot and Murphy, 1926 (1408)	Megaloblastic anemia, monkey, 1932 (1041) Growth factor, rat, 1946 (1075)
	Reproductive failure, rat, 1922 (600) Coagulation defect, fowl, 1935 (684) Biochemical defect, bacteria, 1951 (1267)

(1) 1915 Differentiation between fat soluble (A) and water soluble (B) (23)
(2) 1922 Differentiation between vitamins A and D (534)
(3) 1926 Differentiation between heat laible and stable B group (904)
(4) 1938 Differentiation between riboflavin and acrodynia factor (905)

VI.

OF THE VITAMINS

NAME	ISOLATION	IDENTIFICATION	SYNTHESIS
Ascorbic acid	1932 (703)	1933 (705)	1933 (704)
Thiamine	1926 (755)	1936 (1480)	1936 (1480)
Vitamin A (2)	1930 (carotene) (1483)	1931 (1484)	1931 (1485)
Vitamin D (2)	1924 (536, 537)	1927 (1489)	------
Nicotinic acid	1937 (850)	1937 (850)	------
Pyridoxine(4)	1938 (905)	1939 (1487)	1939 (1488)
Riboflavin (4)	1938 (905)	1933 (806)	1935 (807)
Pantothenic acid	1939 (866)	1939 (864)	1940 (865)
Biotin	1939 (1013)	1942 (957)	1943 (1486)
Inositol	1940 (1033)	------	------
Paraaminobenzoic acid	1941 (1040)	------	------
Choline	1932 (955)	------	------
Folic acid	1943 (1046)	1946 (1050)	1945 (1049)
Vitamin B12	1948 (1415)	1956 (1076)	------
Vitamin E	1936 (604)	1936 (604)	1938 (605)
Vitamin K	1935 (684)	1939 (685)	1939 (685)
Lipoic acid	1951 (1267)	1952 (1269)	------

45 is the result Apologies are in order for many omissions, yet the scheme may be helpful for a general orientation in present-day physiological chemistry

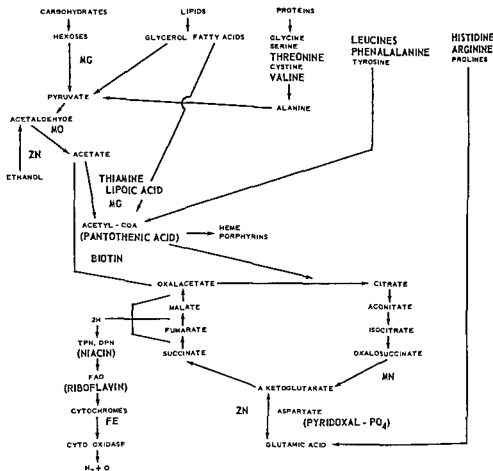


FIGURE 45 SOME INTERRELATIONS OF CERTAIN ESSENTIAL NUTRIENTS IN INTERMEDIARY METABOLISM

VITAMINS A

Recognition[470] of the existence of vitamin A dates from 1913, when two groups of investigators, McCollum and Davis, and Osborne and Mendel, reported that certain fats contain an essential nutrient for rats, without this substance animals failed to grow in normal fashion and exhibited changes in the eyes. Several years later, Steenbock suggested that the active principle, or vitamin A as the material had then been designated, was carotene, a yellow pigment derived from plant and animal tissues During the next ten years the exact identity of vitamin A remained unsettled and in much confusion, in 1930 carotene was shown to be pro-vitamin A. Karrer then worked out the chemical constitution of both carotene and vitamin A and completed the story in 1936 when a pure material was synthesized. Vitamin A_2 was discovered in 1949. There are a number of closely related substances (carotenes) which behave as pro-vitamins, of these, the most potent is beta-carotene.

The carotenes are converted into vitamin A as they are absorbed through the intestinal wall.[471] This newly formed material, together with ingested vitamin A, is stored in the liver As in the case of the other fat-soluble vitamins, bile and/or pancreatic juice facilitate absorption from the intestine, vitamin A deficiency may develop when these secretions are diminished or absent.[472] Although the major portion of the organism's store of vitamin A is found in the liver, other tissues also contain varying amounts which may be demonstrated by chemical or histological studies. The following values indicate the tissue concentrations of vitamin A (in British Units per gram) as determined by chemical procedures on rats·[473] liver, 40,000, kidney, 50, lung, 450, adrenal, 2500, heart, 1, spleen, 2, pancreas, 25, thymus, 12, brain, 0 3, muscle, 0 5; and blood, 2 0.

The fluorescent properties of vitamin A have been utilized to study the distribution of this nutrient under the microscope. When formalin-fixed sections of tissues are viewed through a source of ultra-violet light of wave length 328 millimicrons, a fading, green fluorescence is observed, this is interpreted to be due to the presence of vitamin A Such an histochemical test is not as sensitive as ordinary chemical determinations, since the technique fails to reveal the vitamin in tissues where chemical analyses indicate that it is present.[474]

Although, as we shall shortly see, the chief known function of vitamin A in the mammalian organism is to maintain the integrity of certain important epithelial structures, its biochemical role in achieving this end is

45 is the result. Apologies are in order for many omissions, yet the scheme may be helpful for a general orientation in present-day physiological chemistry

CARBOHYDRATES
LIPIDS
PROTEINS
HEXOSES
GLYCEROL, FATTY ACIDS
GLYCINE
SERINE
THREONINE
CYSTINE
VALINE
LEUCINES
PHENALALANINE
TYROSINE
HISTIDINE
ARGININE
PROLINES
MG
PYRUVATE
ALANINE
ACETALDEHYDE
MO
ZN
ACETATE
ETHANOL
THIAMINE
LIPOIC ACID
MG
ACETYL COA
(PANTOTHENIC ACID)
HEME
PORPHYRINS
BIOTIN
OXALACETATE
CITRATE
MALATE
ACONITATE
2H
FUMARATE
ISOCITRATE
TPN DPN
(NIACIN)
SUCCINATE
OXALOSUCCINATE
FAD
(RIBOFLAVIN)
MN
A-KETOGLUTARATE
CYTOCHROMES
FE
ZN
ASPARTATE
(PYRIDOXAL - PO_4)
CYTO OXIDASE
GLUTAMIC ACID
H_2 + O

Figure 45 Some Interrelations of Certain Essential Nutrients in Intermediary Metabolism

VITAMINS A

Recognition[470] of the existence of vitamin A dates from 1913, when two groups of investigators, McCollum and Davis, and Osborne and Mendel, reported that certain fats contain an essential nutrient for rats, without this substance animals failed to grow in normal fashion and exhibited changes in the eyes. Several years later, Steenbock suggested that the active principle, or vitamin A as the material had then been designated, was carotene, a yellow pigment derived from plant and animal tissues During the next ten years the exact identity of vitamin A remained unsettled and in much confusion, in 1930 carotene was shown to be pro-vitamin A. Karrer then worked out the chemical constitution of both carotene and vitamin A and completed the story in 1936 when a pure material was synthesized. Vitamin A_2 was discovered in 1949 There are a number of closely related substances (carotenes) which behave as pro-vitamins, of these, the most potent is beta-carotene.

The carotenes are converted into vitamin A as they are absorbed through the intestinal wall.[471] This newly formed material, together with ingested vitamin A, is stored in the liver As in the case of the other fat-soluble vitamins, bile and/or pancreatic juice facilitate absorption from the intestine; vitamin A deficiency may develop when these secretions are diminished or absent.[472] Although the major portion of the organism's store of vitamin A is found in the liver, other tissues also contain varying amounts which may be demonstrated by chemical or histological studies. The following values indicate the tissue concentrations of vitamin A (in British Units per gram) as determined by chemical procedures on rats [473] liver, 40,000, kidney, 50, lung, 450, adrenal, 2500, heart, 1, spleen, 2, pancreas, 25, thymus, 12, brain, 0 3, muscle, 0 5, and blood, 2 0

The fluorescent properties of vitamin A have been utilized to study the distribution of this nutrient under the microscope. When formalin-fixed sections of tissues are viewed through a source of ultra-violet light of wave length 328 millimicrons, a fading, green fluorescence is observed, this is interpreted to be due to the presence of vitamin A Such an histochemical test is not as sensitive as ordinary chemical determinations, since the technique fails to reveal the vitamin in tissues where chemical analyses indicate that it is present.[474]

Although, as we shall shortly see, the chief known function of vitamin A in the mammalian organism is to maintain the integrity of certain important epithelial structures, its biochemical role in achieving this end is

completely obscure. On the other hand, as a result of the investigations of Wald,[475] a great deal is known concerning the relation of vitamin A to visual processes. What bearing the reactions to be summarized below might have on the metabolic activities of cells in general remains for future studies to determine

The red pigment of the retinal rods is called rhodopsin. Rod vision is effective in dim light (scotopic). Rhodopsin breaks down under the influence of light to two compounds: vitamin A aldehyde (retenine$_1$) and a protein, opsin This reaction initiates a nerve impulse. The retenine$_1$ (vitamin A aldehyde) is in the form of the *trans* isomer. This is changed to *trans*-vitamin A by the action of alcohol dehydrogenase and cozymase. For the resynthesis of rhodopsin, the vitamin A must be in its *cis* form. How this reaction is accomplished is not clear. New *cis*-vitamin A is doubtless obtained from the circulation and oxidized to *cis*-retinene$_1$ (*cis*-vitamin A aldehyde) which combines with opsin to form rhodopsin and thus completes the cycle.

Cone vision, i.e., that for color discrimination and high light intensity, is dependent on a similar group of carotene substances; a different protein, photopsin, is present instead of opsin. These metabolic pathways of vitamin A in the retina may give some inkling of what takes place in other cells, since the oxidation of *cis*-vitamin A to *cis*-vitamin A aldehyde leads to a much more biologically-active material.

As a result of the studies of Wolbach and Howe and others, a deficiency of vitamin A has been shown to affect the integrity of many epithelial tissues, as well as bones and teeth Individual tissues vary in their sensitivity to vitamin A deprivation.[476]

Microscopic changes in epithelial structures have been described in the rat,[477] guinea pig,[478] fox,[479] mouse,[480] monkey,[481] rabbit,[482] dog,[483] pig,[484] horse[485] and in cattle.[486]

As interpreted by Wolbach,[487] specific changes are found in those epithelia whose cells "have a secreting (chemical) function in addition to the role of a covering layer and whose functioning cells are without power to divide." Epithelial cells comprising the following organ systems have been reported to be affected:

a. *Digestive system:* parotid, submaxillary, sublingual and all accessory glands of the tongue, buccal cavity and pharynx; ducts of the pancreas.

b. *Respiratory tract.* nares, sinuses, Jacobson's organ, larynx, trachea and bronchi.

c. *Genito-urinary system:* renal pelvis, ureter, bladder, urethra, epididymis, prostate, seminal vesicles, coagulating glands, uterus, oviducts, glands of vulva and vagina.

d. *Special senses* eyes, including cornea and accessory glands (harderian, intra- and extraorbital and tarsal).

e *Miscellaneous:* thymus

The reason for the involvement of and the degree of damage to various epithelial tissues is not clear No relationship has been found as yet between the chemical and/or microscopic distribution of vitamin A and the occurrence or non-occurrence of tissue changes An explanation based on embryological grounds is untenable, nor does the order in which various organs are affected by the characteristic keratinizing metaplasia give any clue Wolbach[487] has epitomized the pathogenesis of the epithelial lesions as "atrophy of the epithelium concerned, reparative proliferation of basal



FIGURE 48 VITAMIN A DEFICIENCY

Trachea, rat. *A* Normal epithelial lining of trachea *B* and C Tracheas from rats placed on vitamin A-deficient diet. Columnar cells have been replaced by partially and fully keratinized cells In C there are numerous leukocytes infiltrating between the epithelial elements H and E all (x 500)

cells and growth and differentiation of the new products into a stratified keratinizing epithelium."

If one takes the trachea as an example, microscopic examination reveals that the initial change consists of atrophy of small groups of columnar cells. The decrease in cell size is at the expense of the cytoplasm; the nucleus does not appear to change its dimensions. Such foci spread to embrace large portions of the epithelial lining. Next, small, synctial-like masses of cells, derived from the atrophic elements, appear and proliferate so as to undermine the overlying atrophic columnar cells. Such cell groups rapidly develop into a highly keratinized type which spreads. Thus, as the deficiency progresses, the entire trachea comes to be lined by metaplastic epithelium whose keratinization results in the accumulation of a detritus of cornified material in the lumen Although an increased growth activity of all epithelia was postulated by Wolbach, this view is questioned by Friedenwald and his associates [488] Quantitative studies of mitotic activity of healing wounds in the cornea of vitamin A-deficient rats reveal that the overall mitotic rate for each thousand basal cells is reduced by 30 per cent from the normal and the speed of the mitotic cycle is likewise inhibited. Such observations furnish the first quantitative evidence on this fundamental point.

When vitamin A is administered, repair is extremely rapid and diffuse. In contrast to the focal distribution of the initial changes which is seen when the animal is placed on a deficient regimen,[489] all cells composing the basal layers, which are apparently analagous to the stratum germnativum of the skin, have the power to proliferate. After these structures regain their normal columnar form, the overlying strata, which have seemingly irreversibly differentiated, are sloughed off and removed by autolytic phenomena The tracheal epithelium thus regains its normal morphological appearance.

Some mention must be made of the relationship of the changes in the epithelium of certain specific tissues to the subsequent course of events In the respiratory tract, for instance, the normal ciliated epithelium lining the trachea and bronchi is replaced by keratinizing cells Consequently, the continuous streaming of surface material towards the pharynx is abolished. Since this protective mechanism of the host has been eliminated, pulmonary infections are a common accompaniment of vitamin A deficiency. In the urinary tract, renal and cystic calculi have frequently been observed in animals chronically deficient in vitamin A. Such stones result from the inspissation of keratinized material derived from the lining of the renal pelves and bladder.

Lesions of the eye are of particular importance, especially from the diagnostic standpoint Xerosis and keratomalacia were the first manifesta-

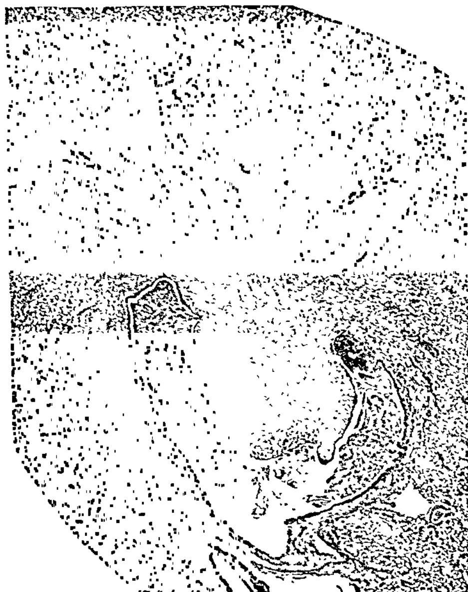


FIGURE 47 VITAMIN A DEFICIENCY

Kidney, rat Note pelvis is filled with debris and ultimately would undoubtedly become obstructed No changes in the renal parenchyma are found at this time. H. and E (x 25).

FIGURE 48. VITAMIN A DEFICIENCY

[illegible] f the pelvic
[illegible] epithelium
[illegible] H. and E.
(x 200).

tions of vitamin A deficiency to be described in man (page 355). The corneal epithelia of deficient rats,[490] rabbits[492] and mice[491] show the characteristic metaplasia seen elsewhere. Vascularization of the substantia propria also occurs but is interpreted to be a secondary phenomenon, which results from the keratinization of the epithelium in association with infection.[492] It is interesting to speculate on how much the corneal changes may be related to vitamin A deficiency and how much they may be secondary to a diminution of the secretions of the ocular glands, because of obstruction of the latter's ducts. Keratitis occurs when the secretion of tears is abnormal; Sjogren's syndrome in the human is a good example.[493]

The corneal changes in vitamin A-deficient mice have recently been studied utilizing the electron microscope.[491] Such observations furnish a picture of the intimate structural details of the basal cells with their nuclei, mitochondria and cytoplasmic components. In the cells of the "intermediate

region," large dense granules are found about the nucleus These granules, presumably "keratohyaline," appear to be related to the mitochondria.

An early manifestation of vitamin A deficiency is nyctalopia, this defect led to extensive studies of the relationship of vitamin A to normal visual processes. Night blindness has been observed physiologically in animals[494] and, of course, in man When severe degrees of vitamin A deficiency are produced in rats, anatomical changes are found in the retina[495] Alterations which are first seen in the visual neuroepithelial cells, the rods, then progress in order through the outer nuclear layer, the outer molecular layer and the inner nuclear layer. The ganglion cells, themselves, are unaffected, even in severely deficient animals The outer segments of the rods, which have degenerated, are capable of regeneration following therapy, if, however, more extensive changes have occurred treatment is ineffectual It will be recalled that the rat, for the most part a nocturnal animal, has very few cones, too few to appear often in sections, so that the reaction of these struc-



FIGURE 49 VITAMIN A DEFICIENCY

Tongue, rat Note large cystic structure filled with keratinized debris This has formed because the normal lining epithelium has become keratinized and the orifice of the duct is obstructed. Smaller cysts are seen on either side. H. and E. (x 25).

tures to vitamin A deficiency has not been described. These changes in the retina may not be due to the direct effect of vitamin A deficiency but rather the result of increased intracranial pressure, which will be referred to below.

Despite extensive changes in the epithelia in other areas, relatively few cutaneous alterations have been ascribed to a lack of vitamin A in experimental animals. In the human, however, Frazier and Hu[496] have described a rough, dry skin which microscopically shows hyperkeratosis and hyperkeratotic plugs in the hair follicles (page 359). For over a decade such changes have been interpreted as pathognomonic of vitamin A deficiency. The specificity of these lesions has recently been questioned on both theoretical as well as experimental grounds.[445, 497]

Sullivan and Evans[497] have called attention to the present concept of the pathogenesis of the lesions of vitamin A deficiency: atrophy followed by metaplastic epithelial hyperkeratinization. They find it difficult to imagine how such changes can occur in the skin since this structure is, of course, already keratinized It thus appears that metaplastic keratinization cannot occur unless the epithelium is atrophic. To produce this picture a vitamin A-deficient ration which was essential in all other respects, particularly the B group and the essential fatty acids, was concocted. Grossly and microscopically no dermal lesions appeared when rats were placed on this diet When, however, the ration was made deficient in other factors, especially the heat-stable B components, changes did develop. For example, rats were given adequate vitamin A, but inadequate amounts of the B group until marked deficiencies of the latter vitamins were present. Vitamin A was then withdrawn and the animals were continued on maintenance levels of the B complex. Microscopic examination of the skin before vitamin A therapy was stopped revealed an atrophic, single layered, epithelial covering. Following the withdrawal of vitamin A, some return to normal thickness was found More important, however, extensive keratinization occurred in the epithelium, especially that of the hair follicles. Such lesions have a superficial resemblance to the changes described by Frazier and Hu[496] in the human. The skin has been studied in mice.[525]

Extensive investigations have been reported concerning effect of vitamin A deficiency on the male germinal epithelium and on reproduction in the female rat. When male rats are placed on a diet deficient in vitamin A, atrophy of the germinal epithelium occurs fairly rapidly, according to Mason[498] more rapidly than similar morphologic alterations which result from inanition. The difference is apparently one of degree since in both inanition and vitamin A deficiency some of the basal cells persist. As a result, when food intake is increased or vitamin A is restored to the diet a rapid return to normal follows, which is unlike the irreversible change

which may be produced in the testis as a result of vitamin E deficiency (page 163).

The female exhibits an alteration in the vaginal smear which is characterized in markedly deficient rats by a continuous cornification of the cells.[499] As a result, the estrous cycle in such animals cannot be interpreted. *In moderate deficiency states one encounters periods of partial cornification*, which can be interpreted as meta and diestrous. Periods of complete cornification doubtless coincide with proestrous and estrous.

Studies of the effects of castration and estrogen administration on the uterine mucosa of vitamin A-deficient rats show that the ovary is necessary for keratinization to take place, since this change is absent in animals which have no gonads When castrated deficient females are treated with estrogen, typical keratinizing metaplasia is found [500] Thus estrogen would appear to have some role in evoking the metaplastic change in non-ovariectomized vitamin A-deficient animals

It has been shown that tissue cultures of the vagina respond to vitamin A [501] *When explants were maintained for six days on normal media the* epithelium keratinized If the medium was supplemented with vitamin A, keratinization was inhibited *and the secretory character of the cells* was retained. On the other hand, a dose of vitamin A which was sufficient to prevent keratinization in the normal medium was unable to stop this change when estrogen was added

The vitamin A-deficient female is capable of normal ovulation, implantation and endocrine activity However, depending on the severity of the deficiency, further reproductive function is impaired [497] Death of the fetus occurs *in utero*, such fetuses may be either resorbed or expelled Gestation may be prolonged, a few young may be born alive, only to die shortly thereafter Mason interprets the primary change as occurring not in the embryo proper, as in vitamin E deficiency, but rather as a result of alteration in the lining of the reproductive tract For at the junction *of the fetal and maternal* tissues one finds localized areas of infection and necrosis in which bacteria have been stained *Such foci of destruction of tissue interfere* with the nutrition of the embryo There may be, too, a bacteremia of the embryo, a possibility which has not yet been *fully* explored *It would* be most interesting to culture the uterine cavities of pregnant vitamin A-deficient females to determine the prevailing bacterial flora

The vitamin A-deficient female rabbit exhibits infertility as a result of changes in the ova which lead to a reduction in the number of viable young per pregnant doe [42, 503] This is due to resorption and abortion. Changes in the fetus and in the placenta have been described grossly.

In their early studies of vitamin A deficiency, Wolbach and Howe[477] noted impairment of epiphyseal bone formation and interpreted this to be

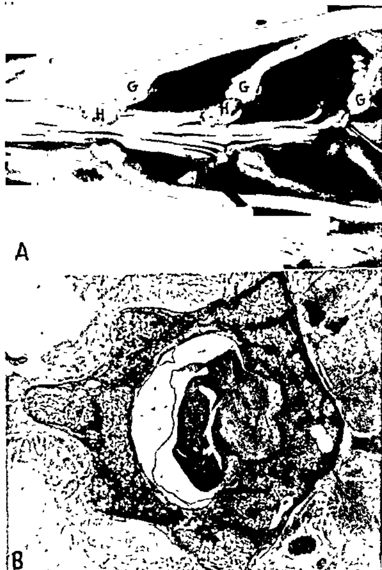


FIGURE 50 VITAMIN A DEFICIENCY.

Skeleton, rat *A* Nerve roots show deformities due to herniation through the vertebral foramina. *B* Cross section of a vertebra in which the herniation is better seen (Courtesy of Dr. S. B Wolbach and the *Archives of Pathology*)

a manifestation of the effects of general inanition which their animals exhibited. At about the same time neurological signs, together with lesions in the nervous tissues, began to assume a prominent place in the syndrome of experimental vitamin A deficiency. Mellanby,[508] and others, had studied and reported degeneration of the cranial and peripheral nerves, together

with lesions in the gray and white matter of the brain and spinal cord. It was postulated, therefore, that vitamin A had a specific effect on nervous tissues, despite the fact that there was no agreement whatsoever as to the pathogenesis or the distribution pattern of the lesions In 1941, Wolbach and Bessey[509] clarified the matter by stating that they were forced to "the paradoxic conviction that the genesis of the nerve lesions of vitamin A deficiency requires an essentially normal rate of growth of a normal nervous system and that mechanical injury, the result of a disproportion between the central nervous system and its bony enclosure, is the explanation." This interpretation is based on several important observations In the first place, lesions can only be produced in young, actively growing animals When rats are placed on a vitamin A-deficient regimen after a certain critical age, neurological manifestations fail to appear When the dietary vitamin A content of weaning animals is restricted, evidence of involvement of the nervous tissue appears with regularity during the eighth week Then, too, the pattern of the lesions has no homogeneousness, neither in a single animal nor when several rats are compared, one with another The explanation for this was found after careful dissection of the nervous tissues within their bony coverings[509] The cerebellum may be found herniated into the foramen magnum Multiple herniations of the cerebrum and cerebellum into the dural venous sinuses at the site of the arachnoidal villi are observed There is usually an over-crowding of the spinal canal by its contents so that the spinal cord is distorted and the nerve roots herniate into the intervertebral foramina and erode the vertebral bodies That these phenomena are a result of vitamin A deficiency alone has been conclusively proved, since disturbances produced by inanition or other specific nutritional deficiencies affect the rate of growth of skeletal and nervous tissues alike Furthermore, that the bone is at fault, rather than that there is an overgrowth of nervous tissue, can be shown since the growth of the latter is normal, in addition the regenerative capacity of the axon is unimpaired

Wolbach and Bessey's[509] observations clearly show that vitamin A has a specific effect on endochondral bone formation Wolbach was unable to detect any specific or characteristic effect on this phase of osteogenesis. *The changes appear to be non-specific and resemble those seen in any bone* which has stopped growing as a result of lack of calories or nutrients other than copper, vitamin D or ascorbic acid However, Wolbach felt that appositional bone formation continues in the shaft and elsewhere until inanition supervenes.[510] Thus a clear dissociation in the growth of the cartilage cell and the osteoblast is demonstrated The effects of vitamin A deficiency on the metabolism of the rat's epiphyseal cartilage has been studied by following the uptake of labeled sulfur by the growing cells[511] A decreased rate of incorporation of S^{35} was found, this is remedied by vitamin

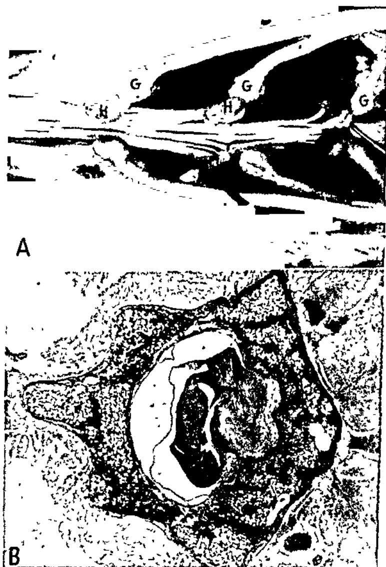


FIGURE 50 VITAMIN A DEFICIENCY

Skeleton, rat. *A* Nerve roots show deformities due to herniation through the vertebral foramina. *B* Cross section of a vertebra in which the herniation is better seen. (Courtesy of Dr S. B Wolbach and the *Archives of Pathology*)

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changes produced in the various layers of the retinas of rats on a vitamin A-deficient diet have been described above (page 000) to increased intracranial pressure. Similar evidence is presented to account for hydrocephalus observed in vitamin A-deficient rabbits[519]

Because of epithelial origin of the teeth it is not surprising to find that deficiency of vitamin A profoundly affects their growth The studies reported in rats and guinea pigs are all in agreement and the underlying



FIGURE 51 VITAMIN A DEFICIENCY

Teeth, rat Note wide band of dentin under the enamel (empty space) A characteristic proliferation of odontoblasts is also seen (Courtesy of Dr Paul E. Boyle)

A administration. The non-specific effect of manition was not well-controlled in these studies, however.

That vitamin A has a potent effect on bone growth has been even more conclusively demonstrated by Wolbach's observations in various species subjected to the effects of large amounts of vitamin A.[510, 512, 513] When toxic doses of the vitamin are administered, the bones become so extremely fragile that numerous fractures result. Bone growth is greatly accelerated so that "it is possible to get the equivalent of a year's growth in a ten to fifteen day period "[512] Wolbach interpreted this action of vitamin A as an acceleration of "remodeling sequences in conformity to normal growth pattern. The remodeling takes place in spite of a retardation of linear growth of bone and is correlated with accelerated epiphyseal cartilage sequences. Those sequences retarded or suppressed in vitamin A deficiency grotesquely and dramatically accelerated by the excess."[512]

The effect of vitamin A on explanted bone rudiments has been studied.[514] Marked alterations are seen in the cartilage; the bone of the shaft becomes increasingly rarefied so that at high doses the explant may virtually disappear by the tenth day.

Besides the cessation of bone growth which occurs as a result of vitamin A deficiency, certain other osseous changes are encountered Mellanby,[508] who has interpreted virtually all the neurological changes on the basis of bony overgrowth, has described hyperostoses about the periotic labyrinth and in certain of the foramina of the skull in dogs. Wolbach and Bessey[503] noted such changes in the inner ear; narrowing of the optic foramina has been observed in calves.[515] The significance and cause of these localized outgrowths of bone are not clear; the subject requires further investigation.

From the studies of the nervous tissues in rats, it is to be expected that an increase in intracranial pressure occurs when these and other animals are placed on vitamin A-deficient diets. In deficient calves a quantitative rise in cerebrospinal fluid pressure has been observed.[516, 517] Ophthalmoscopic examination may reveal papilledema. Cerebrospinal fluid pressure values may increase from the normal of 70 millimeters of water to as high as 240 millimeters. A further manifestation of increased intracranial pressure in such animals is the development of cysts in the hypophysis These are not encountered in normal animals They are observed in the posterior lobe and cause compression of the gland which leads to atrophy and necrosis. Measurements of the cysts have not been given; one, however, is said to have contained .75 milliliters of fluid.[518] In cattle, blindness has been observed but it is not entirely clear how much of a role each of the following factors play: increased intracranial pressure, narrowing of the optic foramina by bony overgrowth, and degeneration of the retina. Microscopic

Extensive studies of the young born to vitamin A deficient female rats have been carried out by Wilson and Warkany.[504, 505, 506, 507] The multiple defects which have been encountered have led to the designation of the distribution pattern as a "syndrome" The areas which are primarily involved are. (1) the eye, (2) the genito-urinary tract, (3) the diaphragm, and (4) the cardio-vascular system. Anomalies in other areas, including the lung, are observed, though infrequently.

The specific ocular defects which have been observed[504] include, post-lenticular fibroplasia, coloboma or incomplete closure of the choroidal fissure, eversion and abnormal folding of the nervous layer of the retina, and other less common changes such as cataracts and absence of lids Among the genito-urinary organs[505] renal changes such as fusion or ectopia are also seen Disturbances in the development of the genital ducts may be prominent In the cardio-vascular system, defects of the aortic arch are common, these include double arch, distally arising right subclavian, truncus arteriosus communis, absence of ductus arteriosus and right sided arch. Interventricular and truncoconal septal defects were the most common malformations in the heart itself[506]

When females which have been maintained on a deficient diet are treated on different days of their pregnancy,[507] a progressive reduction occurs in the numbers of young exhibiting malformations This may be correlated with the time at which treatment was instituted. The conclusion which

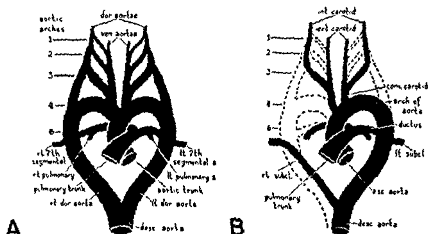


FIGURE 52 VITAMIN A DEFICIENCY

Malformations, cardiovascular system A Normal or hypothetical pattern of aortic arches B Double arch-double ductus L4-R4-L6, a "vascular ring" anomaly. (Courtesy Dr J Warkany and *The American Journal of Anatomy*)

changes appear to be well-established.[520, 521, 522] Before describing the alterations which occur in the rat's incisor, it would seem advantageous to review briefly the normal development of this structure.[522] Growth of the incisor results from the proliferation of a group of epithelial cells at the base of the tooth. Such odontogenic epithelium differentiates into ameloblasts which form enamel on the outer or labial surface of the tooth and cementoblasts which form the cementum which is deposited on the lingual and lateral margins. Odontogenic epithelium is also responsible for the organization of mesenchymal pulp cells into odontoblasts of a "polarized" or functional type; the latter cells then lay down dentine which, while building up, is calcified and is responsible for the growth of the tooth. In the normal growth of the rat's incisor one may then expect an orderly sequence as follows: (1) Proliferation of ameloblasts. (2) Differentiation of ameloblasts (3) Differentiation of odontoblasts. (4) Formation of dentine matrix. (5) Calcification of dentine and enamel

In vitamin A-deficient animals the first and principal change is found in the odontoblasts It will be remembered, however, that such cells are organized by the odontogenic epithelium; hence, although in the early stages of the deficiency the latter cells are morphologically not abnormal, the physiological stimulus they ordinarily provide appears to be inadequate. The odontoblasts do not differentiate or arrange themselves in normal fashion and in consequence dentine is formed irregularly and in varying amounts. The lingual dentine is thin, while that deposited over the labial surface is thicker than normal. It has been suggested that masticatory stresses may lead to this difference[522] The odontoblasts show varying degrees of development, being more poorly differentiated proximally than distally, possibly because the former cells are younger and more deficient than those which are farther away and, therefore, older.

In the early stages of vitamin A-deficiency the odontogenic epithelium appears to be normal morphologically though not so physiologically, later, profound anatomical changes are observed. The cells exhibit such a lack of differentiation that virtually no recognizable ameloblasts can be found. Consequently, there is a great reduction in the deposition of enamel, as a result enamel hypoplasia is a prominent manifestation of advanced vitamin A deficiency Since the odontogenic epithelium does not stop its proliferative activity, cords of undifferentiated epithelium invade the pulpal tissues where they form nests of cells. Some of these are able to stimulate the neighboring mesenchyme to abortive efforts of dentine formation and in this fashion numerous concretions may be formed. Loss of the yellow pigment of the incisor teeth of rats has been described when rations deficient in vitamin A are employed.[523] In the rat and guinea pig all of the above changes are reversible following adequate treatment with Vitamin A.[520,521]

VITAMINS D

The skeletal manifestations which characterise rickets have been recognized from earliest times However, not until the middle of the seventeenth century was the disease shown to be a clinical entity Chemical and pathological studies of the bones of animals and humans were made during the last century (page 361), such observations culminated in Pommer's morphological observations which were published in 1885 [527] Pommer outlined the broad principles of the pathological changes in human rickets Subsequent workers have only confirmed and amplified his conclusions.

Although many attempts had been made to produce rickets in the experimental animal, not until the end of the second decade of this century was evidence presented that pathologic alterations similar to those seen in the human could be achieved with regularity in the laboratory. In 1918, Edward Mellanby [528] announced that he had been able to produce rickets in dogs and that the disease was "primarily due to a lack of an accessory factor in the diet" The first ration used by Mellanby consisted of oatmeal, rice, salt, and whole milk. This regimen was changed from time to time, at the end of his experiments, bread, linseed oil, yeast, orange juice, salt, and separated milk were the ingredients The presence of rickets was determined by the external appearance of the dogs, x-ray changes, the chemical composition of the bones, and histological study of the skeletal tissues Rickets could be prevented or cured by the administration of cod liver oil. In addition to an "anti-rachitic vitamine" in the diet, Mellanby believed that certain adverse environmental conditions, particularly confinement in cages, contributed to the development of skeletal changes [529]

Meanwhile in America, two groups of investigators were carrying out experiments which were to clarify further our understanding of rickets. In 1921, McCollum, Simmonds, Parsons, Shipley, and Park [530] reported the production of rickets of rats which had been placed on a variety of diets which were deficient in calcium, phosphorus, or fat-soluble vitamin A. They proceeded to demonstrate the importance of the levels of calcium [531] and/or phosphorus [531] in the diet, for, if rickets was to be produced, one of these elements must be lacking Meanwhile, Sherman and Pappenheimer had independently announced the production of rickets in rats by a diet deficient in phosphorus [532] The addition of phosphate protected against the development of skeletal changes.

The Johns Hopkins investigators then demonstrated that the inclusion of cod liver oil in the diet led to the deposition of lime salts in the bones of rachitic rats [533] and that cod liver oil contains a curative substance not

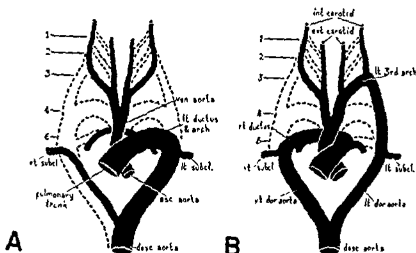


FIGURE 53. VITAMIN A DEFICIENCY.

Malformations, cardiovascular system A. Left ductus as arch, L-4, with distally arising right ductus (Courtesy Dr. J. Warkany and *The American Journal of Anatomy*)

may be drawn is that such malformations are determined during the period of active organ formation rather than at an earlier period such as has been found for other agents which produce defects in the embryo

The various effects of naturally occurring vitamin A deficiency in man are described elsewhere (page 355). Well-controlled studies of experimental vitamin A deficiency have been carried out in the human for periods ranging from six and one-half to twenty-five months.[524] The subjects were twenty men and three women, between the ages of nineteen and thirty-four years. The diet consisted of natural foodstuffs which supplied no more than 70 I.U. of beta-carotene daily Only two useful criteria of vitamin A deficiency emerged: plasma concentration vitamin A and dark adaptation values. Within three months the average value of carotenoids in the blood dropped from 150 I U per 100 ml of blood plasma to about 40 I.U. In time there was also reduction in plasma vitamin A levels. In four individuals the value fell below 50 I.U per 100 ml of plasma. In only three of sixteen subjects was there a marked deterioration of dark adaptation. A number of tests were employed in this study but none were effective in demonstrating any changes from the normal. Slit lamp studies of the eye and careful examination of the skin failed to reveal any alterations. The conclusion was reached that "the observations, therefore, do not support the view that, in the condition of the present experiment, lack of vitamin A was a causal factor in the development of follicular hyperkeratosis"

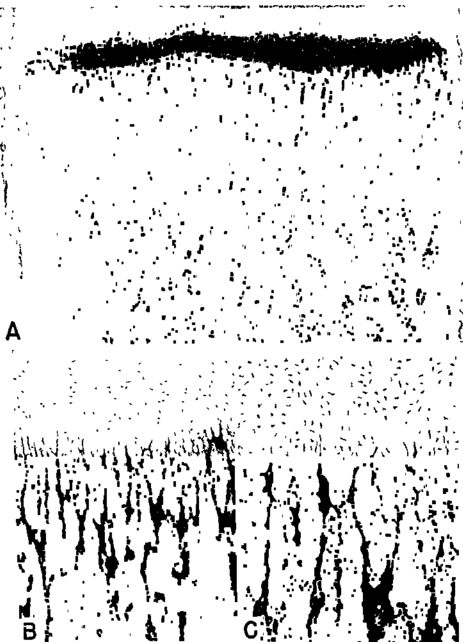


FIGURE 54 NORMAL BONE

Human A Low power (x 15) of costochrondal junction from a seven month old male dying of acute dysentery Note the small, undifferentiated cartilage cells lying in haphazard fash[illegible]
as the cartilage
In *B* and *C*, [illegible]
matrix is seen,
to the cartilage-shaft junction.

present in butter fat. The existence of two fat-soluble vitamins was demonstrated when oxygen was bubbled through cod liver oil, a procedure which destroys vitamin A while the antirachitic potency remains intact.[534]

While such studies were going on attention was directed to Huldschinsky's[535] observation that ultra-violet light has a curative effect on clinical rickets The entire story was brought to a close when Steenbock[536] and Hess[537] simultaneously showed that, when various substances were irradiated, antirachitic properties appeared. The precursors of these active materials were demonstrated to be ergosterol and cholesterol.

Of the many forms of vitamin D which have now been discovered[538] two, activated ergosterol (viosterol or calciferol) and activated cholesterol (7-dehydro cholesterol), are the most important; both are used extensively in the prophylaxis and therapy of rickets Aside from these dietary forms of vitamin D, the organism is able to obtain adequate amounts of antirachitic substance from the activation by sunlight of the provitamin in the skin. *Hess and Weinstock*[539] *proved thus by feeding human or calf skin to rats* on a rachitogenic diet. While non-irradiated skin has little or no healing effect, dermal tissues which have been radiated with ultra-violet rays *in vitro* do have antirachitic power. These observations show conclusively why ultra-violet irradiation is so important in the cure and prophylaxis of rickets and also help explain the seasonal[1306] and geographical distribution of the disease.[1319]

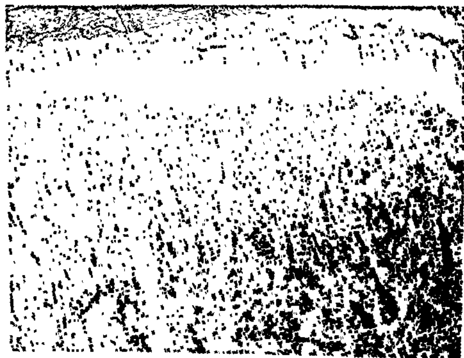
As might be expected, following the isolation of several active forms of vitamin D experimental data began to accumulate concerning the mode of action of such potent factors. Today, although some broad generalizations can be offered, little information is available concerning any specific effects of vitamin D on cellular mechanisms. Before discussing the few data which are at hand, it would seem appropriate to define the morphologic criteria of rickets, whether the disease is produced by a deficiency of calcium, phosphorus, and/or vitamin D, and the biochemical defects which may be encountered. With such a background we shall be in a better position to evaluate the experimental evidence dealing with the mode of action of vitamin D. But we shall first have to go farther afield; for if the pathologic changes which characterize rickets in the skeleton are to be made intelligible, a brief discussion of normal osteogenesis must be presented: the anatomical aspects first, followed by pertinent data on the composition of cartilage and bone, to be concluded by what little is known of the calcification mechanism.

Virtually all the bones of the mammalian skeleton are preceded by cartilagenous replicas Only certain bones of the skull arise directly from a connective tissue matrix. Bone formed from cartilage is termed endochondral; hence, this type of skeletal growth is termed endochondral bone

calcium and phosphorus appear in the organic matrix and true bone is thus formed. Much of it is soon destroyed in order to lighten the structure.

As we shall see later, it is advantageous to divide normal bone growth into three general events (1) Growth of cartilage, (2) Bone formation and destruction, and (3) Deposition of inorganic salts in cartilage and bone matrices.[542]

The epiphyseal cartilages are composed of cells and matrix [544] The latter is said to consist in large part of protein, collagen (see page 186), and a carbohydrate, chondroitin sulfuric acid, which is made up of molecules of glucuronic acid and acetyl-galactosamine-sulfuric acid, *arranged alternately in long chains* [797] *The matrix of cartilage is apparently elaborated by the* cells themselves One can follow the incorporation of radioactive sulfur (S^{35}) in the cell and its subsequent localization in the extracellular matrix [561 562] The turnover of chondroitin sulfuric acid would appear to be rapid So, too, the metabolic activity of cartilage with respect to organic



T ... onal epiphyseal cartilage and underlying bone
N ... age cells and trabeculae of bone beneath. The marrow cells
ar ... and are found up close to the cartilage *H and E. (x 60).*

formation. Its characteristics are that each bone is preceded in the embryo or fetus by a replica formed of cartilage cells. If one examines such a structure, the cells which compose it are found to be in varying degrees of maturation; the most advanced or adult cells are present in the central region, for instance, the mid-portion, in the case of the cartilagenous anlage of a long bone.[540] Here one finds a zone of large (hypertrophic) cells, in which certain chemical changes are occurring. At the periphery of this cell mass, beneath a sheath of perichondrial cells a change also takes place; for these elements promote the formation of an organic fibrous matrix in which inorganic salts soon deposit. Hence, a bony collar is soon formed about the region of hypertrophic cartilage cells. Blood vessels and connective tissue cells soon break through the bony collar and begin to excavate the mass of hypertrophic cartilage cells. A marrow cavity is thus initiated. Cartilage cells at either end (now the epiphyses) continue to proliferate, reach an hypertrophic stage, and are invaded by capillaries. The bony shell has now become the shaft, or diaphysis, which continues to form new bone on its external surface as a result of the activity of the cells which are now called the periosteum.

Certain more intimate details of the growth of cartilage now concern us, particularly those at the junction between the cartilage and bony shaft, a region which is called the metaphysis. One can trace the growth of cartilage cells from small elements through various stages of development to their final or hypertrophic form.[541] Alterations in the lipid, carbohydrate and protein content of these cells also occur. Such changes are doubtless related to the great proliferative activity of these cells which manifests itself by the rate of growth of the epiphyseal cartilage. For instance, the lower end of the femur of a rat increases .18 mm. in length per day.[595] It has been estimated that the complete cycle of growth, maturation, and death of the cells in the upper epiphysis of the tibia of the rat takes only thirty to forty-five hours.[594]

Changes are seen in the enzyme content of the cells; for instance, alkaline phosphatase activity appears in the hypertrophic cells and the matrix surrounding them.[542] Intense dehydrogenase activity is found, which, however, has no particular distribution with relation to the cycle of growth of the cells.[563]

The most prominent alteration is seen in the matrix between the cartilage cells. Here, between the rows of hypertrophic cells, inorganic salts are deposited. A sort of honeycomb is made by the spaces occupied by dead or dying cartilage cells; into these, capillaries grow. The blood vessels are accompanied by osteoblasts which deposit the organic matrix of bone, or osteoid, on the spicules of calcified cartilagenous matrix. Simultaneously,

bone crystals just noted must be deposited. How is this procedure brought about? Two main areas, humoral and local, would appear to be of importance. More is known of the first than the second mechanism

Knowledge of the importance of the humoral factors in calcification stemmed from studies of healing rickets in children and in experimental animals. In 1918 Howland and Kramer[547] pointed out the importance of the serum concentrations of calcium and phosphorus in the pathogenesis of rickets If a product in terms of milligrams per cent calcium and phosphorus was below thirty, rickets was sure to be present, if between thirty and forty the disease might be expected, while if the product was forty or above the bones could be assumed to have their normal content of inorganic salts Studies on experimental animals in which rickets had been produced by diets low in calcium and/or phosphorus soon indicated that the development of the disease could be correlated with the serum levels of calcium and phosphorus. Moreover, the effects of healing *in vivo*,[548] as well as *in vitro*,[549] also demonstrated the importance of the humoral concentrations of calcium and phosphorus on their deposition in cartilage The latter studies indicated that slices of epiphyses from rachitic rats would calcify in solution containing appropriate concentrations of calcium and phosphate Various procedures such as changing the concentrations, boiling the cartilage, et cetera, inhibited the calcification process

The exact mechanism whereby calcium and/or phosphorus combines with the organic moieties of cartilage and bone remains unknown Some have felt that a local accumulation of phosphorus was decisive Robison[594] invoked his famous phosphatase theory to achieve high local concentrations of phosphate ions as a result of hydrolysis of phosphate esters Others[555] have looked upon binding of calcium, possibly by chondroitin sulfuric acid, as of importance More recently, evidence for the role of the collagen molecule has been brought forward[545] At the moment we just don't know how this very important process comes about However, we shall continue to look upon the calcification mechanism as a process mediated by cells Rather too much emphasis has been placed on the physical aspects of the process, as a result of studies utilizing the *in vitro* technique. We feel that this procedure has been abused in past years and in the hands of some workers has ceased to be physiological. Exactly what the roles of glycogen[517] phosphatase,[542] enzymes of the Krebs cycle,[563] et cetera,[597] have to do with the calcification mechanism in cartilage remain to be determined Whether the mechanism is the same in bone matrix must also be decided.

From the above brief discussion of the dynamic aspects of the ion relationships to the bone crystal, it must be obvious that the skeleton is not particularly static During growth of the experimental animal or of man, growth of cartilage, bone formation and bone destruction are actively going

carbon compounds labeled with C-14 may be studied[560] and found to be active.

Bone matrix consists of collagen and a polysaccharide which has not been as clearly defined chemically as has that of cartilage.[544 733]

At the present time the chemical nature of bone salt is fairly well settled.[545 546] The basic structure of the bone micro-crystal is hydroxyapatite, which belongs to compounds composed of calcium, phosphorus and hydroxyl ions having a certain spatial or lattice arrangement, which is common to the whole series of materials having different molecular ratios of these ions. The chemical formulation for hydroxyapatite is $3(Ca_3(PO_4)_2) \cdot Ca(OH)_2$. Crystals of bone mineral, which are composed of aggregates of hydroxyapatite, are small having dimensions in the neighborhood of 200 x 100 x 20 A. The small size of such crystals allows for a variable stoichiometry and helps explain the presence of certain other ions in bone.

Much of this distribution is governed by the ionic radii of the atom in question in relation to the lattice structure and its hydration shell. Ions of strontium, radium and calcium can exchange with calcium already present; so, too, fluoride or phosphate may substitute for the latter ions already in bone. In addition, other ions: sodium, magnesium, uranyl, and lead, may be adsorbed on the surface of the crystal in place of calcium and carbonate or citrate may be adsorbed in lieu of phosphate. Finally, certain ions may be more loosely fixed in the hydration shell; these include potassium and chlorine.[545] In recent years bone has come to be looked upon as a vast reservoir for the storage and ready supply of a number of inorganic ions. Elsewhere, for instance, this phase of the metabolism of sodium has been pointed out (page 33).

The most extensive changes take place with respect to the primary constituents of hydroxyapatite, that is calcium and phosphorus. Twenty years ago, Hevesy[131] showed how rapidly and completely newly-administered radioactive phosphorus (P^{32}) must be taken up by skeletal tissues. This observation initiated a vast number of studies dealing with the dynamic equilibrium of numerous ions with bone, all of which have shown how labile this tissue is, at least as far as its inorganic composition, is concerned particularly with respect to calcium[559, 584] and phosphorus[596] One can view these changes going on in several ways: (1) by formation of unit crystals of hydroxyapatite, (2) by recrystallization, (3) by surface exchange or absorption, and (4) by diffusion into the hydration layer of the bone crystal.[545]

The growth of cartilage and the activities of bone cells, the osteoblasts, lead to the formation of their respective matrices. In order to give these organic components, particularly the latter (osteoid), more stability, the

The mechanism whereby the cells of the intestinal epithelium effect the transfer of calcium into the blood stream is not known. Presumably, as will be noted below, Vitamin D plays a role.

A certain minimal amount of calcium must be present in the diet if positive balance is to be maintained. Wide variations from individual to individual are found. Moreover, many exogenous and endogenous factors, not all of which are clearly understood affect calcium balance. The problems which are encountered in studying this phase of calcium metabolism are many. Long term studies of intake and excretion are necessary if valid data are to be obtained. The importance of the proportion of "fecal endogenous" calcium has been stressed repeatedly in recent years.[552, 554]

Calcium exists in the blood plasma in two forms, ionized and bound. Utilizing a biologic assay method, about one half of the 10 mg. of calcium is found to be ionized or in the diffusible state.[553] The un-ionized portion of plasma calcium is bound to the albumin fraction of the plasma proteins.

As shown by studies employing radioactive calcium (Ca^{45}), the absorbed calcium is removed from the blood stream by the skeletal tissues. This is an extremely rapid process, beginning immediately after Ca^{45} is introduced into the blood stream or intestinal tract; one half of the absorbed dose may be removed in a matter of minutes.[559]

Under normal conditions relatively little calcium is reexcreted into the lumen of the intestinal tract. Most is eliminated by the kidneys, though this is not a particularly large amount, since even though large quantities pass through the glomeruli, much of the calcium in the fluid presented to the tubular epithelial cells is reabsorbed.

There are thus three factors which guard the calcium homeostasis of the organism: (1) intestine, (2) skeleton, and (3) kidneys. Of these three, the skeleton appears to play the most important role, hence, when one finds alterations in the metabolism of calcium, the skeleton is likely to be the seat of disease.

The mechanism for the absorption of phosphorus[551] is no more understood than that for calcium. As far as is known all of the phosphorus in the circulating plasma is freely diffusible. The main regulatory mechanism for phosphorus is the kidney, which is controlled in part by the parathyroid glands. The role of the skeleton is important yet in this respect calcium and phosphorus differ somewhat since the metabolism of the latter is so much more important as a part of tissues other than bone.

During the past forty years rickets has been studied in experimental animals by a number of investigators.[164, 527, 529, 530, 531, 532, 533, 536, 537, 538, 571, 572, 574, 598] The morphological alterations are, in general, well-understood. The chemical changes in the structure of cartilage and bone are not at all clear and much needs to be done before the entire story is elucidated. In



FIGURE 56. NORMAL BONE.
Cortex, human. Microradiograph of ground section of portion of cortex of adult human bone to show Haversian systems of various sizes. *Note especially the different degrees of density of the osteones. Some are less mineralized (darker) than others. The intervening compact bone is most dense* (× 75).

on In the adult organism, since growth of the epiphyses has ceased, one will see only alterations in the osseous tissue. But here, too, evidence is not lacking for active turnover The Haversian systems or osteones are continually being formed and broken down. Moreover, differences in their mineral content can be demonstrated by appropriate techniques [550]

Since the integrity of the bones is so dependent on their content of calcium and phosphorus, it is necessary to discuss briefly some of the normal mechanisms for the regulation of the metabolism of these two components of hydroxyapatite

The absorption of calcium which is taken into the gastrointestinal tract is modified by a number of local factors.[551, 552] These include the pH of the intestinal contents, presence of excessive amounts of anionic compounds, amount of protein and/or amino acids present, and concentrations of fatty acids Calcium absorption may be modified by any one or more of these factors as is noted in Table VII where appropriate references are included.

between the rows of hypertrophic cartilage cells; this deposit seems to guide the ingrowth of capillaries into the holes left by the degenerating cartilage cells Osteoblasts then form osteoid on these spicules of calcified matrix. In rickets, the initial change at the cartilage shaft junction is failure of lime salts to be deposited in the cartilaginous matrix substance. Such defects are exhibited in tissue sections by an absence of deep blue-staining material (calcium salts) between the rows of cartilage cells. The extent of such defects is dependent upon the severity of the metabolic disorder One may use other techniques such as staining with silver nitrate or microradiography to demonstrate the defects.

Coincident with this defective lime salt deposition the zone of mature cartilage cells begins to increase in width. When the disease has progressed for a time, the histological picture is characterized by a broad zone between the multiplying cartilage cells and the shaft, the so-called rachitic metaphysis. This is composed of tongues of cartilage which extend down toward the shaft and which are separated from one another by collections of capillaries or "vascular bushes" In other words, at some point blood vessels have been able to penetrate the cartilage while in other situations, due perhaps to compression of cartilage or differences in lime salt deposition, capillaries find it impossible to erode the cartilage In addition, this zone contains trabeculae made up of uncalcified cartilage matrix upon which osteoid is being deposited. These, of course, result from groups of cartilage



FIGURE 58 RICKETS

Phosphatase activity, rat. Epiphyseal cartilage from rat on a low-phosphorus diet A Control stain to show widespread zone of hypertrophic cartilage cells. B Phosphatase reaction to show intense staining of entire area which indicates enzyme activity throughout.

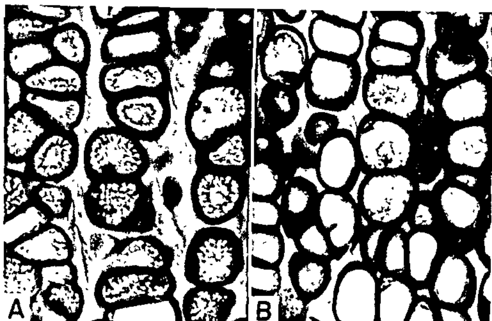


FIGURE 57. RICKETS

Epiphyseal cartilage, rat *A* Most recently hypertrophic cells from rachitic epiphysis shown in Figure 18 The cells contain nuclei and cytoplasm with radiating strands *B* Cells from hypertrophic area which has been present for a long time. Note difference in size, irregular arrangement and apparent absence of material in cells Toluidin blue (×575).

the following account we shall draw on the reports just cited, together with material which we have studied ourselves.

The rat has been used most often to study the pathogenesis of rickets. In this species pathologic alterations may be produced in the skeleton as a result of either calcium (page 44; Figures 17, 18 and 19) or phosphorus (page 52, Figures 22 and 23) deprivation. Usually vitamin D is excluded from the diet as well, although this is not absolutely necessary.

When a young growing animal is placed on an appropriate diet, the initial changes, which may be seen after only a few days, are found in the cartilage. These alterations may be epitomized as follows: (1) failure of lime salts to be deposited in the cartilage matrix between the rows of hypertrophic cells and (2) failure of these cells to be invaded and destroyed by the capillaries.

In the normal growth of the cartilage plate on the shaft, the cartilage cells multiply and those nearest the diaphysis arrange themselves in rows with the largest and most adult cells nearest the capillaries advancing from the shaft. Lime salts are deposited in the cartilaginous matrix substance

levels of each will fall In addition to these changes in the concentrations of serum calcium and/or phosphorus, the alkaline phosphatase activity of the serum is found to be elevated.[565]

The effects of alterations in the intake of calcium or phosphorus on serum levels are manifested by changes in the chemical composition of the bone One can modify the Ca.P ratio of bone over a range by altering to the concentrations of these materials in the diet[566] or by adding other materials in excess, as will be noted below.

Changes in the excretion pattern of calcium or phosphorus are naturally to be expected. If the diet is low in calcium content, much of the phosphorus in the ration will not be absorbed and hence will be excreted Conversely, on a low-phosphorus diet large amounts of calcium are lost in the urine and feces[163] This phenomenon shall be returned to later when the mode of action of vitamin D is discussed

So far, only the importance of changes in the calcium and phosphorus contents of the diet has been mentioned. The amounts of these materials in rations may be optimal, yet rickets can develop as a result of a number of conditioning factors. Most of these studies have been carried out on rats,



FIGURE 59. RICKETS

Healing, rat *A* Epiphyseal cartilage of rat on low phosphorus diet which had received injection of phosphate twenty-four hours before Note deposition of black staining (silver nitrate) material in upper zone of hypertrophic cells *B*. Section of rachitic epiphysis to show distribution of glycogen in area where lime salts will deposit. (PAS stain)

cells being cut off by capillaries and then coated with osteoid, doubtless an attempt to strengthen such a region.

As might be expected changes are also occurring in the shaft, about the trabeculae, and along the endosteal and periosteal surfaces. In rickets osteoblastic activity is not affected unless the organism is suffering from growth arrest as a result of other factors such as non-specific inanition. Therefore, as one would expect, osteoid, the organic matrix of bone, is deposited upon pre-existing bony trabeculae in normal fashion. However, since general, and perhaps local, factors are not propitious, calcium and phosphate salts are not deposited in this osteoid. It will be recalled that ordinarily the deposition of inorganic salts occurs practically simultaneously with the appearance of osteoid; in rickets, however, deposition of lime salts in bone matrix is either retarded or completely lacking. In the usual tissue sections stained with hematoxylin and eosin, osteoid may be recognized as a band of light pink-staining material of varying width which coats much of the cortex and trabeculae of the shaft. In such histological preparations bone usually has a bluish-gray tint. The contrast between bone and osteoid may be accentuated if more elegant techniques are used, such as those which employ undecalcified sections stained with silver nitrate or studied by microradiography. When examining a bone under the microscope one should always look at the shaft to ascertain whether osteoid is present in abnormal amounts. One must, of course, bear in mind that the amount of normal, or physiological, osteoid varies depending upon the age and the species from which the specimen is derived. In the normal growing rat, for instance, osteoid is virtually never seen.

The healing phenomena in rickets have been studied in experimental animals since the very beginning.[548] Inorganic salts deposit *in vivo* in the matrix about the cells which have most recently become hypertrophic It is here that glycogen is present [567] The deposition is dependent on the concentrations of calcium and phosphorus in the plasma. Healing can be effected by the administration of inorganic phosphate parenterally if a low phosphorus type rickets is being studied.[570] The exact mechanisms which are entailed in the deposition of inorganic elements are not known. They are undoubtedly the same as those occurring in the cartilage of the normal organism. Histochemical studies of healing phenomena have been interesting and need to be expanded.[597]

What are the biochemical defects in experimental rickets? These depend on whether the skeletal alterations result from a deficiency of calcium or phosphorus. If the former element is lacking from the diet a reduction in serum calcium levels will be found On the other hand, if the phosphorus content of the diet is reduced, the levels of serum phosphate will be diminished If the intake of both calcium and phosphorus is restricted the serum

tion of lime salts in it. Whether this delay represents some chemical immaturity of the matrix which is not propitious for calcification is not known. At any rate, bands of osteoid of varying widths are found along the bony trabeculae of the normal growing animal. Such layers are called "physiological osteoid" To explain this one can conceive of either more osteoid being formed than the calcification mechanism can keep up with, or a defect in the humoral concentrations of calcium and phosphorus. Two situations come to mind with respect to excess osteoid formation. new matrix production as a result of fractures in the normal animal[570] or new matrix production in healing scorbutus[728] In both situations osteoid formation outstrips calcification, though we can never be sure that the osteoid has not reached the normal "state of calcifiability." Growth is necessary if rickets is to develop, rickets may be made to heal if growth is retarded.[569]

Any number of factors may affect the absorption of calcium and/or phosphorus by the cells of the intestinal mucosa Some of the mechanisms which may be studied in the experimental animal are· (1) dietary lack, (2) inadequate absorption of calcium due to lack of vitamin D and (3) inadequate absorption of calcium and/or phosphorus as a result of the presence of other ions or complexing agents

Any ions or organic materials, which may combine with calcium and/or phosphorus in the intestinal lumen to form insoluble or poorly absorbable compounds, can lead to rickets In the case of calcium, such material consists of phosphate,[574, 1147] citrate,[573, 1148] oxalate,[575, 576, 1148] phytate,[508] protein,[577] and certain amino acids, particularly lysine,[577] and fatty acids[552] In the case of phosphorus, such materials include calcium,[574] beryllium,[578] iron,[579, 580] lead,[581] aluminum,[579] magnesium,[582] thallium[584] and manganese[583]

The importance of the pH of the intestinal contents on calcium and phosphorus absorption has been recognized for many years.[573] This mechanism is related to the solubility of calcium phosphate which is increased in an acid medium When rickets or osteomalacia occur naturally the situation becomes even more complex (page 361)

We are now in a position to discuss the mode of action of vitamin D.[585] The main function of vitamin D, though how this action is effected in unknown, is to increase the absorption of calcium by the cells of the intestinal mucosa. Evidence for this is based on several types of data. In the intact, vitamin D-deficient organism fecal losses of calcium are usually large, such deficits may be decreased by the administration of vitamin D. The alterations in phosphate excretion and retention parallel those of calcium. More evidence was presented some years ago by Nicolaysen who studied the absorption of calcium from isolated loops of intestine of the rat Calcium was found to disappear at a faster rate from the isolated loops of vitamin

TABLE VII

THE PATHOGENESIS OF EXPERIMENTAL RICKETS

I Genetic[568]

II Disturbance in Balance of Matrix Production and Deposition of Inorganic Elements

- (1) Healing fractures[570]
- (2) Healing scurvy[178]

III Disturbance in Intestinal Absorption of Calcium and/or Phosphorus

- (1) Calcium
  - (a) Dietary lack[571, 572]
  - (b) Change in pH of intestinal contents[573]
  - (c) Formation of insoluble complexes (aa) phosphate,[574, 1147] citrate,[573, 1149] oxalate,[575, 576, 1148] phytate[504]
  - (d) Protein and amino acid content of diet[577]
  - (e) Fatty acids[552]
  - (f) Vitamin D lack
    - (aa) Dietary
    - (bb) Absence of bile
    - (cc) Impaired formation in skin
- (2) Phosphorus
  - (a) Dietary lack[184]
  - (b) Change in pH of intestinal contents[573]
  - (c) Formation of insoluble complexes
    - (aa) calcium[574]
    - (bb) beryllium[578]
    - (cc) iron[579, 580]
    - (dd) lead[581]
    - (ee) aluminum[579]
    - (ff) magnesium[582]
    - (gg) manganese[583]
    - (hh) thallium[584]

although, as will be related later, similar situations may apply to rickets occurring naturally in man and animals. A summary of the pathogenesis of experimental rickets is found in Table VII. Here it will be noted that the development of skeletal changes can be broken down into three main categories. (1) Genetic, (2) Disturbance in the balance of matrix production and deposition of inorganic elements in it, and (3) Factors affecting the intestinal absorption of calcium and/or phosphorus

Some years ago Streeter, Park, and Jackson[568] reported on breeding experiments in rats in which strains having a high or low susceptibility to rickets had been obtained. This was accomplished by studying the severity of the disease in a number of animals placed on a standard rachitogenic diet and in breeding the animals according to the degree of rickets which they evinced. This is a beautiful example of the importance of genetic factors on the pathogenesis of deficiency disease.

In the normal growth of the rat or other experimental animal, there is a slight lag between the appearance of bone matrix (osteoid) and the deposi-

this structure can be detected. There is no enamel hypoplasia in rats, although in the guinea pig severe hypoplasia of the enamel has been reported[572] when these animals are placed on a low calcium-high phosphorus diet containing no vitamin D. Thus in experimental animals there does not appear to be entire agreement, though it will be noted the calcium and phosphorus concentrations of the diet were reversed and this may explain the presence or absence of enamel hypoplasia. Pointed studies on the teeth using diets of known composition while changing the calcium and phosphorus ratios and total concentrations as employed by Shohl[574] in the study of bone are certainly needed

As might be expected, the bony supporting structures of the teeth show characteristic changes similar to those of the bones just described above. Wide osteoid borders are found on the trabeculae of the alveolar bone.[598]

D-deficient rats as compared with those receiving the vitamin.[590, 591] More recently studies with the isotope, Ca^{45}, have indicated an increased absorption in the presence of vitamin D.[592] It may be concluded that vitamin D has a profound effect on the absorption of calcium in the species which have so far been studied, including man. The rat is able to absorb sufficient amounts of calcium and phosphorus for its needs if the dietary levels of these two elements are optimal. Considerable increase in the amounts of calcium absorbed can be effected by vitamin D administration. The absorption of phosphorus is secondary to the effect of vitamin D on calcium.

Are there any other physiological effects of vitamin D? Several have been postulated, of these most attention has been given to two: the role of vitamin D on phosphorus excretion by the kidney and a direct effect on the activities of the osteoblast. Evidence for the effect of vitamin D on the metabolism of phosphorus has been derived from experiments on rachitic puppies, in which a decrease in resorption of phosphate was found.[595] Whether a similar effect operates in the normal remains to be demonstrated.

Nicolaysen's contention is that a lack of vitamin D leads to the presence of excess osteoid in bone independent of its ash content.[585] We feel that evidence is not yet sufficient to warrant a sweeping conclusion that vitamin D deficiency (in the rat) leads to specific effects on the bones, other than those associated with alterations in serum calcium and phosphorus concentrations As a matter of fact excess amounts of vitamin D lead to abnormal amounts of osteoid.[586] The effect of excess strontium is difficult to explain The administration of this element leads to excess osteoid production.[599]

The vitamin D content of serum and tissues may be assayed.[587, 588] The bioassay procedure is a laborious one. Other methods may not be as satisfactory

Changes in the teeth in rachitic animals are less complex than those occurring in bones mainly for the reason that the former are never resorbed. When young rats are placed on a rachitogenic diet (high calcium, low phosphorus, no vitamin D), the first and most prominent change is in the incisors where a line of disturbed calcification appears in the dentine; this has been called the "calciotraumatic line"[589] It is found in the dentine and represents the first response of the organism to the effects of the rachitogenic regimen Almost immediately, too, there is a retardation in the formation of predentine together with a pronounced disturbance in the calcification of all the dentine which is formed, this material is not homogeneously basophilic but is stippled by an irregular deposition of calcium salts. Calcification of the cementum is likewise retarded. The changes in the molars are similar but not of such a severe degree. Although there are cystic alterations in the enamel organ before it undergoes atrophy, no other abnormalities in

VITAMINS E

(*Alpha-tocopherol and its Homologs*)

In 1922, Evans and Bishop[600] reported the existence of a new dietary factor which was found to be necessary in order to insure normal reproduction in rats, this "X" factor was considered to be distinct from then known vitamins A, B, and C Mattill *et al.*[601] soon showed that testicular degeneration occurred in rats whose diets were deficient in this material. A third important manifestation of a deficiency of vitamin E, as the factor had by then been named, was reported in 1928 by Evans and Burr[602] who noted the development of paralysis in young rats born to mothers on vitamin E-depleted diets Ten years passed before Olcott[603] showed that this "paralysis" was not neurogenic in origin, as had been considered, but was due to necrosis of striated muscle fibers

During the period in which these morphological changes were being studied in vitamin E-deficient animals, work was pushed on the identification of an active principle. In 1936 Evans and his group[604] announced the isolation of certain alcohols from wheat-germ oil, one of which had strong vitamin E-like properties This alcohol was named alpha-tocopherol (tokos —childbirth, phero—to carry) Soon after, Karrer and his associates[605] succeeded in synthesizing the first of several biologically active products

Very early in investigations on the chemical properties of vitamin E, its strong antioxidant activity was recognized As a result of these studies of the oxidation of fat tissue,[606] the metabolism of muscle,[607] and the destruction of other lipid nutrients *in vivo*[608] [609] current theories of the mode of action of alpha-tocopherol have placed its role as an anti-oxidant in the foreground, though its precise locus in enzymatic reactions has yet to be fully elucidated.

The body fat of rats which are raised on a diet deficient in alpha-tocopherol is very susceptible to oxidation[606] When the missing factor is administered, body fat is stabilized, oxidation does not take place. The relationship of alpha-tocopherol to muscle metabolism *in vitro* will be discussed in detail below It has been suggested that alpha-tocopherol "acts as a brake on the oxidative mechanism primarily of skeletal muscle and in its absence oxidative processes in muscle run riot."[607] Studies of the interrelations of vitamin A and alpha-tocopherol have shown that the inclusion of the latter in a diet containing vitamin A prevents the destruction of vitamin A in the gastrointestinal tract.[608] Somewhat similar experiments demonstrate that other fats such as cod liver oil destroy alpha-tocopherol and thus

evidence of damage to the ovum or its membranes can be detected until the middle portion of pregnancy, that is, at the time of implantation The earliest evidences of any untoward effects are found during the eighth day, when the ectodermal cavity fails to appear. Further indications of deranged development are manifested during the following days. As a result of inadequate growth of ectoderm, the ectoplacental and amniotic cavities fail to form, so, too, retarded development of fetal mesoderm and of the blood islands is found In addition, irregular development of the liver is prominent, together with an absence of blood cells in the heart and large vessels. By the twelfth day, the development of the deficient embryo is a full day behind its controls, on the thirteenth day death of the organism is apparent, since the tissues become macerated The entire embryo is then resorbed. Studies of the placenta indicate a marked retardation in its development and failure of invasion by the fetal vessels. Evans et al.[619] have attempted to explain reproductive failure as a result of "starvation and asphyxia", fetal

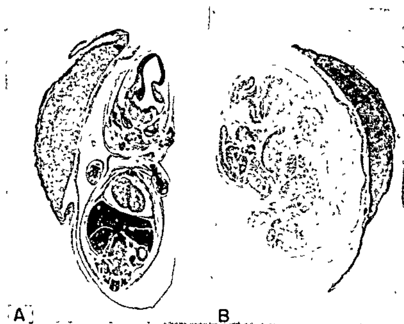


FIGURE 60. VITAMIN E DEFICIENCY.

Embryo, rat Two fetuses removed from females at the sixteenth day of pregnancy A From a normal. B From a vitamin E-deficient female of the same gestation period The placenta of the latter is a little smaller than that of the normal. The fetus in B is dead and is undergoing autolytic changes Note the differences in the color of the liver, heart and cerebral tissues (x 6). (Courtesy of Dr. Karl E. Mason)

produce the pathological lesions characteristic of vitamin E deficiency.[609]

Certain other important observations which throw light on its biochemical activities should be cited. In earlier studies on the metabolism of striated muscle from vitamin E-deficient rats high Qo_2 values were described.[606] To explain this, the hypothesis that vitamin E acted as an inhibitor of succinic dehydrogenase activity was advanced. Further investigations, however, have tended to show that such an inhibiting effect is nonspecific.[610] One of the most interesting contributions has been made by Dam and his collaborators who have shown that methylene blue, a well-known redox dye, will, in appropriate amounts, replace vitamin E in many physiologic processes in the rat. These aspects of vitamin E deficiency will be more specifically described below. Here it need only be mentioned that methyelene blue will replace vitamin E in correcting abnormal hemolysis of erythrocytes,[611] pigmentation of fat and incisor teeth,[612] liver necrosis and pulmonary hemorrhage,[613] storage of vitamin A in the liver,[614] and reproductive activity.[615] These observations are undoubtedly of extreme importance yet their true significance is not clear at the present time.

The participation of vitamin E in the aerobic oxidation of reduced pyridine nucleotide (DPNH) has recently been suggested.[616] We cannot go into the details of these experiments, in which evidence is presented to *indicate that tocopherol is concerned with the activity of cytochromes* b and e. It is hoped that the precise role of vitamin E in biologic systems may be shortly forthcoming

Alpha-tocopherol is absorbed from the intestinal tract like the other fat-soluble vitamins. The importance of normal intestinal secretions, particularly bile, in its absorption has been demonstrated in dogs with biliary fistulas.[617] *Alpha-tocopherol is not distributed in rat tissues as its fat-soluble* properties might indicate. For instance, rather high concentrations may be demonstrated in heart, spleen, and lung, although these tissues contain relatively little fat.[618]

Alpha-tocopherol is necessary for the development of the embryo, for the integrity of the male germinal epithelium, for the maintenance of the metabolism of striated and cardiac muscle *in vitro* and structure *in vivo*, and for the integrity of certain other tissues, such as fat, liver, teeth, and lungs.

The indispensability of this vitamin for the reproductive process has been demonstrated in rats,[619, 620, 621] mice,[622] guinea pigs[623] and swine.[624] Evans and his co-workers[619] studied this phase extensively in the first species where changes are found in the embryo and in its membranes. *Ovulation, the estrous cycle, as well as ovarian and the structure of uterine tissues*, are all normal save for the presence of pigment in the latter; this will be discussed below. Female animals mate normally; *no microscopic*

which Evans places so much emphasis is secondary to loss into the dilated vascular channels.

Degeneration of the male germinal epithelium has been described in the rat[626, 627] and guinea pig[628] but not in the rabbit[629] or mouse[622] although muscular lesions occur in the latter species (see below). Mason[627] divides the sequence of events which may be seen in the rat into several stages as follows. after fifty to one hundred days on the deficient diet there is lysis and fusion of the mature spermatozoa, this debris then finds its way into the epididymis. Following the disappearance of spermatozoa, the spermatids assume a vesicular form and disintegrate Next, the spermatocytes show peculiar nuclear changes with partial liquifaction of chromatin and segregation of this material to form intranuclear crescents. Mason postulates changes in the cell membrane since such cells coalesce to form large, characteristic, multinucleated masses. During this period degeneration of the primary spermatocytes and spermatogonia is observed Although some of the Sertoli cells degenerate, for the most part these cells are not particularly damaged. The end result is a testis whose tubules are atrophic and lined only by Sertoli syncytium. If one gonad of a vitamin E deficient rat is removed fairly early in the deficiency and examined, it may appear perfectly normal under the microscope Nevertheless, even though more than adequate amounts of the missing nutrient are administered, the changes described in the end stage above are found in the opposite one many days later Thus, irreversible injury occurs before it can be detected morphologically This, of course, is quite different from what occurs in the testis as a result of vitamin A deficiency (page 132) or manition (page 13), for repair can always be elicited in these situations

It is now known that the "nutritional muscular dystrophy" of rabbits and guinea pigs which Goettsch and Pappenheimer[630] described in 1931 is due to a deficiency of alpha-tocopherol[631] Lesions have been described in the skeletal musculature of the rabbit,[630] guinea pig,[630] young rat,[632] mouse,[633] dog,[634] cat,[635] mink,[636] monkey,[637] calf,[638 639 640] lamb[641 642] and pig.[643]

Biochemical changes, which will shortly be discussed, occur in the rat's muscle before any morphological criteria of damage appear.[644] The initial histological abnormality, which is found in all species so far studied, consists of swelling and hyalinization of the muscle fibers, these then become completely necrotic Sometimes an increase in the fluid content of the interstitial spaces is found, such fluid may contain enough protein to be stained by the usual procedures Leukocytic infiltration has been described as a prominent *feature in most species, together with a marked proliferation* of the sarcolemma nuclei Fat droplets appear, together with globules of a peculiar golden pigment, which will be discussed more fully below. Many of the necrotic muscle fibers become infiltrated with calcium salts, which

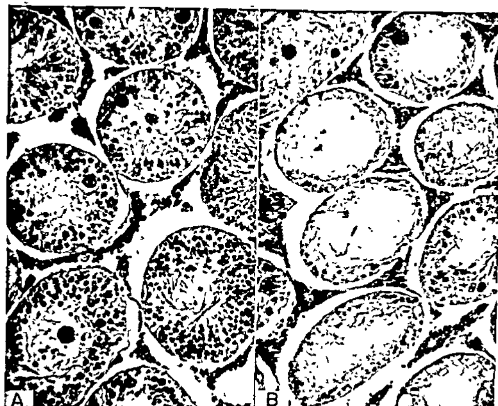


FIGURE 61. VITAMIN E DEFICIENCY

Testis *A* and *B* Various stages in the development of testicular atrophy in animals placed on a vitamin E-deficient diet. Compare with Figure 2A There is decrease in the number of cells lining the tubules, together with the appearance of characteristic giant cells which are especially prominent in *A*. Note decrease and virtual absence of spermatozoa, spermatids, and in some tubules, spermatocytes In *B* giant cells are seen together with a further reduction in the number of germinal elements, in several of the tubules only the Sertoli syncytial cells remain. Eosin-methylene blue stains (x 150). (Courtesy of Dr. Karl E Mason)

nutrition is interfered with because of poor connections between fetal and maternal tissues, and those nutrients which reach the embryo, especially oxygen, do not obtain proper distribution because of inadequate hematopoiesis. Whether such hypothesis is tenable remains to be settled by further investigation, since Mason [625] has postulated a physiological or morphologic defect of the fetal blood vessels as the primary cause of the pathogenesis of the changes observed. The young of female animals given border line doses of vitamin E may show marked dilatation of the blood vessels and extensive hemorrhage into the tissues. This is followed by death of the latter elements Mason suggests that the paucity of blood islands and cells upon

demonstrated in the rat[645] The number of nerve endings returns to normal if there is repair of the muscle fiber. Data on the behavior of sensory endings are not available.

There is great variability among species in the ease which muscle lesions may be produced. Thus, arranged in order of highest susceptibility, one can place the rabbit, guinea pig, hamster, rat, mouse and monkey. A similar situation holds for the heart (page 167), though in cattle myocardial lesions are found early, while changes in the skeletal muscles occur less readily.

In the relatively resistant rat, only scattered necrotic skeletal muscle fibers are seen after this species has been for some time on a vitamin E-deficient regimen If certain other nutrients, such as pyridoxine, protein or vitamin A, are also removed from the tocopherol-deficient diet more extensive muscle lesions are found.[646] Deficiency of thiamine, riboflavin or pantothenic acids in association with a lack of vitamin E have no such effect.

Muscle tissue from rats deficient in vitamin E has been studied by special techniques, interesting data have been obtained Extraction procedures followed by examination in polarized light reveal a loss of positive birefringence before degeneration can be observed by more conventional light microscope techniques.[647] The ordinary birefringent material is thought to be actomyosin, which thus appears to be decreased or lost in muscle fibers from deficient animals. In contrast to the positive birefringent material negatively birefringent substances persist in dystrophic muscle fibers The pattern of distribution which is demonstrated by this technique is similar to that following denervation. Studies[648] of the optical properties of the proteins extracted from dystrophic muscles have revealed a decrease in actomysin content. Actin obtained from muscle tissue showing severe detrophic changes loses it capacity to be activated by various salts. Electrophoretic studies[649] of normal and dystrophic muscle tissues show an increase in the percentages of the more rapidly moving components and a reduction in those which move at a slower rate.

As might be expected, profound disturbances in the chemical composition and physiology of the muscle fibers accompany these morphological alterations Increased oxygen consumption of the muscle tissue occurs *in vitro* before any histological lesions can be demonstrated.[644] In the rabbit[650] the potassium and magnesium contents of the muscle are found to be decreased, while the concentrations of sodium and chloride are increased, the former out of proportion to the latter The percentage of calcium and phosphorus is increased in the muscles of those animals which show histological evidence of calcification In rats, an increase in acid-soluble phosphorus compounds and a reduction in the phosphorylation of glycogen has been noted[651] In rabbits an increase in fat, phospholipid and cholesterol

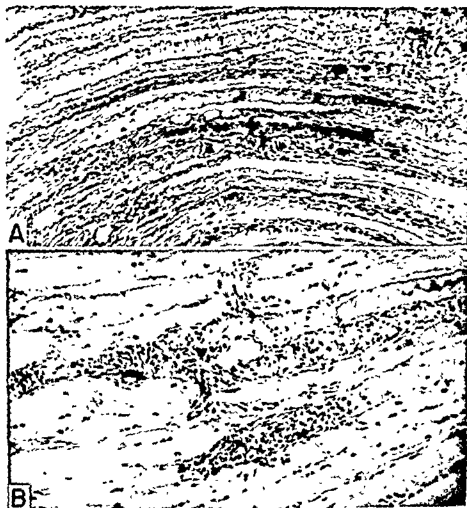


FIGURE 62. VITAMIN E DEFICIENCY.

Striated muscle A Tissue from rabbit showing destruction of fibers with cellular in- [illegible] In several places there are fat vacuoles.

may be identified by appropriate stains. Calcification is especially prominent in calves.[640] Following the administration of alpha-tocopherol there is prompt regeneration of the damaged muscle fibers and an ultimate return of the tissue to its normal appearance in the rabbit and guinea pig Muscle lesions in the rat do not respond well to therapy. Accompanying the degeneration of muscle fibers, a disappearance in motor end plates has been

lesions in that limb. The reason for this is not clear, whether the abolition of motor or sensory, or sympathetic fibers or all is resopnsible has yet to be determined.

In many of the earlier studies of vitamin E deficiency in various species no changes were noted in cardiac muscle. Further investigations have shown, however, that myocardial fibers have become necrotic in a fashion similar to those fibers of striated muscle, on the whole, the process takes longer to appear. Thus, physiological and anatomical changes have been noted in rats,[656] rabbits,[659 660 661 662 663] mice,[664] calves,[640 665] and lambs [666]

In rats which had been on a deficient diet for over twelve months, necrosis of cardiac muscle fibers followed by fibrosis has been observed [658] In such animals, ceroid (page 258) is found in the myocardial fibers and in macrophages in the interstitial tissues. Evidence of destruction of myocardial fibers is present but the process seems to be slowly progressive. The most conspicuous change is the presence of a great deal of connective

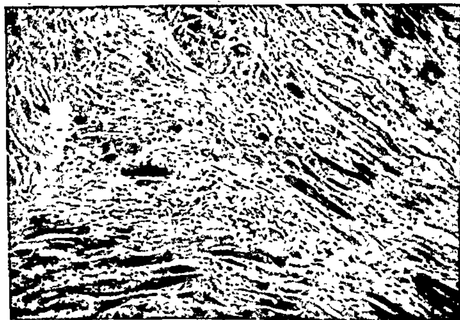


FIGURE 63 VITAMIN E DEFICIENCY.

Heart, rat Myocardium from rat on a vitamin E-deficient diet for over a year Note extensive scarring and disappearance of myocardial fibers. There is, in addition, some cellular infiltration, many of the cells are macrophages containing ceroid pigment There is no evidence of fresh necroses (x 150). (Courtesy of Dr. Karl E. Mason)

concentrations has been observed in the skeletal muscles and, in addition, there is an increase in the blood cholesterol levels.[652] The creatine content of muscle is found to be reduced[653] and there is an increase in the excretion of this substance in the urine, so that the course of the syndrome and the response of a deficient animal to therapy may be followed by studies of urinary creatine. The dystrophy of experimentally produced vitamin E deficiency in calves has been likened to a naturally occurring malady seen in Europe and America[638] This is a form of calf paralysis in which striated and heart muscle are affected. It has been named "*weisses Fleisch*" and is thought to be similar to "stiff lamb disease" which responds to vitamin E therapy[641]

By far the most interesting biochemical change in the muscle tissues of alpha-tocopherol-depleted animals is a marked disturbance of respiration *in vitro*. Increases in oxygen consumption of 200 to 400 per cent were first described by Victor.[654] The studies of Houchin[607, 655, 656] have furthered our knowledge of these metabolic phenomena In the striated muscle from deficient animals of various species the oxygen consumption is as follows: hamster, 240-250 per cent of normal; rabbit, 220 per cent of normal, nursing rat, 160 per cent of normal, grown rat, 125 per cent of normal. Following the oral administration of alpha-tocopherol to deficient hamsters, the QO_2 falls to normal levels in as short a time as twenty-two hours. Studies of biopsied muscle tissues from depleted rabbits to which alpha-tocopherol phosphate had been given intravenously indicate that there is a drop in the QO_2 of 34 per cent in the first hour and of 49 per cent more in the next three hours

In vitro observations of dystrophic muscle slices from rabbits and hamsters show that the addition of alpha-tocopherol to the medium lowers the QO_2 by 40 per cent. Muscle slices from normal animals are not so affected. The succinoxidase activity of dystrophic hamster muscle is found to increase 160 per cent above normal Addition of alpha-tocopherol to the medium decreases the succinate activity toward normal. The effects of alpha-tocopherol appear to be non-specific, hence it cannot be directly implicated in the succinoxidase system.[610] In view of these *in vitro* studies of muscle metabolism it is unfortunate that the data are so inadequate with regard to the total metabolism of the organism. When a group of vitamin E-deficient rats on a purified diet is compared with animals which had received alpha-tocopherol the total oxygen consumption of the latter animals is lower. Unfortunately however, there were no very marked differences between the deficient group and stockfed controls[621] This is a subject requiring further investigation.

Pappenheimer and Goettsch[657] made the interesting observation that complete denervation of an extremity prevents the development of muscular

tion reveals even more pigment in the tissues mentioned above with particularly large accumulations in the fat.[669] The pigment, which is produced as a result of vitamin E deficiency alone and which is intensified by adding cod liver oil to the deficient diet, resembles "ceroid" (page 258) in many respects A beginning of an understanding of the complex mechanism leading to the production of this pigment has been made by Tappel.[670] Hemoglobin, cytochrome or hemin were added to mixtures of cod liver oil and casein. After shaking in air, large increases of a yellow brown pigment were detected, this was thought to result from the catalysis of fat by the iron porphyrin groups. Such a combination of oxidized lipid and protein has been termed co-polymerization The effects of antioxidants on this cod liver oil-protein-porphyrin system were investigated Certain materials were more effective in preventing it than alpha-tocopherol, though the substances which were active are not as readily absorbed as is vitamin E *In vitro* model studies such as these cannot be used to translate what is occurring *in vivo*, although they are extremely provocative.

Pigmentation of adipose tissue has been described in certain animals fed diets containing materials, such as cod liver oil, which might be expected to destroy vitamin E Species so studied include cats,[635] mink[636] and swine[680]

The yellowish brown color of the enamel on the anterior surfaces of the rat's incisor teeth is familiar to all who have worked with this species The pigment, which is found in the enamel, contains iron and is said not to be a porphyrin or lipochrome, but may be a melanin like material deposited by the ameloblasts[523] The disappearance of this pigment from the incisor of the vitamin E-deficient rat has been noted by several workers[671, 672] The iron portion of the pigment may be stained blue in the incisors of normal rats by potassium ferricyanide, the teeth of vitamin E-deficient animals remain unstained As might be expected the iron content of the incisors from such rats is decreased[673] Histological study[674] reveals no enamel hypoplasia, such as is seen in vitamin A deficiency (page 138). Changes in the ameloblasts, such as abnormal accumulations of these cells, cyst formations, and, sometimes, even atrophy, have been described.[675]

The development of acute massive necrosis of the liver as a result of feeding diets low in protein and high in fat content has been ascribed primarily to cystine deficiency (page 98) The fats, such as cod liver oil or lard, which are usually incorporated into such rations contain large amounts of unsaturated fatty acids. The addition of tocopherol to diets which produce necrosis of the liver in rats leads to protection similar to that afforded by cystine (or methionine) administration[676] Hence, it is clear that two distinct factors, cystine and alpha tocopherol, play important roles in the pathogenesis of massive hepatic necrosis Other materials may

tissue separating one myocardial fiber from another; ceroid-laden macrophages are found as well. In contrast to the changes which have been described in the rat's skeletal muscles, no alterations in the lipoid or cholesterol content of vitamin E-deficient cardiac musculature have been observed.[667]

Rabbits deprived of dietary vitamin E, are less resistant to posterior pituitary extracts, yet more resistant to such cardiac glycosides as ouabain and digitoxin[659] In some rabbits electrocardiographic tracings have revealed elevation of the S-T segment and inversion of the T wave in lead II.[660] Microscopic examination[661] of the myocardium reveals changes similar to those which have been found in the skeletal musculature. First there is necrosis with loss of striations of individual fibers; next, nuclear changes, i.e., pyknosis and karyorrhexis are found; these, then, are followed by an inflammatory reaction. Calcification of some of the fibers may occur. The effects of treatment in rats or rabbits have not been investigated.

In calves subsisting on vitamin E-deficient rations or on a regimen in which vitamin E may be destroyed, changes may be found in the heart. The first reports[665] dealt with calves which died suddenly. Electrocardiograms as early as four months prior to death revealed an increase in the P-R interval, this was followed by changes in the QRS complex. Histologically, atrophy and scarring were found in the ventricular walls. A more extensive study in the Ayrshire calf by Blaxter and his co-workers[638, 639, 640] has revealed myocardial involvement as a result of vitamin E deficiency. Electrocardiographic changes have also been noted in vitamin E-deficient lambs.[666]

Chemical studies[663] of the myocardium from normal and vitamin E-deficient rabbits have shown a reduction in creatin phosphate content of the latter group from normal values of 6 1 mg. per cent to .9 mg per cent. No changes were detected in the concentrations of inorganic phosphate, adenosine polyphosphate or glycogen.

Mason and Emmel[658, 668] have carefully studied a curious pigmentation which a number of investigators have noted in many tissues of vitamin E-deficient animals. Small globules of yellowish material are found in the uterine muscle fibers, ovary, interstitial cells of the testis, lymph nodes, spleen, fat, macrophages of the liver, bone marrow, lung, kidney, as well as voluntary and cardiac muscle. The pigment, which is acid-fast, apparently first accumulates in the uterine muscle fibers. It is readily taken up by macrophages, but these cells do not appear capable of digesting it. Tocopherol treatment seems to arrest pigment production but does not appreciably increase its rate of disappearance. When 20 per cent cod liver oil is incorporated in the diet of vitamin E-deficient rats, an intense brown discoloration of the adipose tissue appears grossly; microscopic examina-

VITAMINS K

In 1929, Henrik Dam described a hemorrhagic syndrome in chicks, which had been placed on a diet virtually devoid of sterols.[682] The bleeding tendency was ascribed to "a lack of a factor or factors occurring in cereals"[683] In 1935 Dam presented evidence that the factor was a vitamin which he designated vitamin K ("Koagulations-Vitamin").[684] During the next few years several laboratories carried out extensive studies which led to an elucidation of the chemical nature and finally to the synthesis of a relatively large group of compounds of which 2 methyl-1, 4-naphthaquinone (Nenadione) is the most active[685]

The natural vitamins K are fat-soluble Hence, their absorption is enhanced by bile acids and pancreatic juice Animals in which biliary fistulae have been produced and humans with obstruction of the biliary tract develop the manifestations of vitamin K deficiency[686] Synthetic vitamin K (menadione) is more effective in correcting the coagulation defect in dogs with bile fistulae than are the naturally occurring forms[687]

The distribution of menadione labeled with C^{14} has been studied in dogs and mice[688] Absorption is rapid after intramuscular injection. Traces can be detected in the blood, most is excreted in the urine No appreciable amounts of radioactive material are stored in any tissue After the highest dose (1 mg per 25 gms of mouse) only a trace of activity is detected in the liver and lung

It has been known for some years that vitamin K is concerned with the blood clotting mechanism All of its effects were originally ascribed to a reduction in prothrombin activity As a result of the advances in our knowledge during the past few years of additional factors implicated in the coagulation mechanism, the role of vitamin K must be re-evaluated Previous measurements of "prothrombin time" have not measured the true activity of prothrombin itself The field of blood coagulation is complicated by an expanding terminology, several terms may apply to the same entity Figure 182 is presented in an attempt to provide a simplified version of blood coagulation, the places at which vitamin K affects the various components are indicated It will be noted that vitamin K deficiency leads to a reduction in prothrombin activity[689] This is a measurement of true prothrombin, a protein of known molecular weight which is made by the liver. Vitamin K deficiency is also associated with a reduction in the content of plasma thromboplastin component (PTC, Christmas factor, factor IX).[690] This factor was originally noted to be congenitally lacking in a group of patients, it is of interest that a similar deficiency can come about on a nutri-

also be implicated. The necrosis of the liver associated with the inclusion of large amounts of yeast in the diet is discussed elsewhere (page 101). *In addition to rats, liver necrosis has been produced experimentally in* swine;[677] a naturally occurring form of hepatic necrosis appears to be related to vitamin E deficiency in the presence of large amounts of cod liver oil.[678] In another study alpha-tocopherol has been shown to afford protection against necrosis of the liver produced by carbon tetrachloride administration[679] Hence, one wonders whether the cystine (or methionine) and tocopherol type of hepatic necrosis is due to a pure nutritional deficiency or represents the result of the action of some "toxic" substance with which the liver is ordinarily able to cope.

Several groups of investigators have described extensive lesions in the nervous tissues of vitamin E-deficient animals. Wolf and Pappenheimer[681] have critically reviewed this work and concluded from their own experimental material that "lesions of the central nervous system did not occur in vitamin E-deficient rats at any age." It is of some interest, however, that changes in the lipid content of the brain have been reported in vitamin E-deficient rats. The total lipid and cholesterol concentrations are increased; the free cholesterol portion is said to be elevated.[687]

LIPOIC ACID

As a result of studies on the effects of certain substances on the growth of various types of bacteria, a new biocatalyst has been isolated from liver[1267] and yeast[1268] This material, which is fat soluble, has been called "lipoic acid." Chemically it is characterized as a member of the dithiooctanoic group.[1269]

Lipoic acid has been shown to function with thiamine and coenzyme A in the oxidation of pyruvate. It has been suggested that both thiamine pyrophosphate and lipoic acid are separate coenzymes, or that the two materials make up a single coenzyme, attached to pyruvate oxidase[758]

Evidence for growth promoting activity of this material has been submitted[1270] When rats were studied on purified diets, the addition of lipoic acid increased weight gain No other effects were noted

tional basis Another component of the coagulation mechanism, Factor VII (stable factor, proconvertin), is affected by vitamin K deficiency.[691] Hence, the production of these three materials: prothrombin, plasma thromboplastin component, and Factor VII, appears to be influenced by vitamin K. The biological defect or defects behind those changes are unknown, though some light is being shed on the problem. For instance, evidence has recently been presented that vitamin K may act as part of an enzyme system concerned with the coupling of oxidation and phosphorylation in the liver where it might be assumed these materials are formed [692]

The relationship of vitamin K to the anticoagulant factor of "sweet clover disease," 3,3'-methylenebis (4-hydroxycoumarin) or dicoumarol deserves mention [693] Various compounds having vitamin K activity are effective in counteracting the action of this anticoagulant in rats; the lives of animals fed doses of dicoumarol, which doses are ordinarily toxic, are prolonged when diets containing vitamin K are employed.[694]

Vitamin K deficiency, which may be demonstrated by a reduction of blood "prothrombin" levels, has been demonstrated in several species. The physiological defect of "prothrombin" deficiency is associated with severe hemorrhage, no other changes have been detected in the tissues of vitamin K-deficient animals. The possible relationship of "prothrombin" deficiency to capillary integrity is a subject of great theoretical interest which should be studied further. As Wolbach and Bessey[487] have pointed out, one wonders "if the diminished clotting power of the blood is the complete explanation of the bleeding because it requires the assumption that in ordinary activity, with attendant traumatization, the clotting mechanism is constantly being called into action in normal individuals."

In the rat, hemorrhages occur in numerous areas, though most commonly in the subcutaneous tissues of the lower extremities. Bleeding is also seen in the thymus which may be greatly distended by red blood cells, in the bladder, eye, adrenal, testis, kidney, retroperitoneal tissues and the various serous cavities.[695] A study in the rat has revealed the presence of multiple hemorrhages in the brain.[696] Similar changes are found in the brains of animals born to vitamin K-deficient mothers [697]

A most interesting observation has been reported in pregnant rabbits,[698] when female animals are fed a vitamin K-deficient diet they consistently abort during the early stages of pregnancy, i.e., eight to fourteen days after mating. Microscopic examination reveals the presence of fresh and old hemorrhage in the decidual plates of such animals.

ASCORBIC ACID

The familiar observations by Lind[700] during the eighteenth century plainly indicated that citrus fruit juices contained a substance which protects against scurvy in the adult So, too, when infantile scurvy came to be recognized in the last two decades of the nineteenth century citrus juices were recommended Holt, in the first edition of his textbook (1898) specifies that the treatment of scurvy, "is remarkably simple· to discontinue all proprietary foods and condensed milk and give an abundance of fresh cow's milk, beef juice, orange juice or other fresh fruit, and, in cases that are over a year old, potatoes" [701] In 1907, Holst and Frolich[702] were able to produce a disease in guinea pigs which was similar in all respects to infantile scurvy, whose pathological characteristics had been clearly defined during the previous ten years (page 387) With a test animal on which to assay the antiscorbutic activity of various materials, one might have expected that progress would have been rapid It was not, and twenty-five years were to elapse before King and Waugh[703] announced the chemical nature of a biologically active material or vitamin C, as the antiscorbutic material had come to be called The successful synthesis[704] and the structure[705] of vitamin C were soon reported Shortly thereafter, the vitamin was named ascorbic acid.

Although scurvy was probably the first clearly defined nutritional disease and active antiscorbutic material was the first of all the vitamins to be crystallized and synthesized, we know less of the biochemical role of ascorbic acid in the organism than that of any of the other vitamins Save for the guinea pig, primates and the human, all species are able to synthesize sufficient ascorbic acid to supply their needs. The rat appears to form ascorbic acid from d-glucose *via* an intermediate synthesis of d-glucuronic acid[706]

Ascorbic acid is absorbed from the intestinal tract and is then widely distributed by the blood stream to the tissues As determined by chemical analyses or by histochemical methods, certain organs have higher concentrations than others By the former technique ascorbic acid is found in greatest quantities in the adrenal glands, where in the guinea pig concentrations average 75 mg per gram. The vitamin C content of other representative guinea pig tissues has been reported as follows (in mg. per gram)· liver, 0 10, brain, 0 14, kidney, 0 087; heart, 0 088, skeletal muscle, 0 032; testes 0 18[707] Studies of the distribution of the vitamin by histochemical methods confirm the chemical analyses and aid in a more precise localization of ascorbic acid in the cells Such techniques take advantage of the

have implicated ascorbic acid in yet another important physiological process, the secretion of intraocular fluid. The vitamin is one of a group of reducing substances which is stored in the stroma of the ciliary body. When ascorbic acid is restricted by dietary means, the content of this substance falls rapidly, so that after 24 to 48 hours no reducing substance can be titrated in the aqueous. A coincident decrease in the secretion of the intraocular fluid occurs, so that the pressure falls. It was postulated that the secretion of the aqueous is dependent on differences in oxidation-reduction potential between the ciliary stroma and its epithelium Ascorbic acid thus acts as a "moderator in a redox chain connecting the oxidase activity of the epithelium with the dehydrogenase activity of the stroma." In this way water is tranferred across the epithelial-stroma barrier

The first study of experimental scurvy in guinea pigs was reported in 1907 by Holst and Frolich[702] The colored plate in their article is the first of many illustrations of a specific change in the skeletal tissues of guinea pigs,which has become familiar to most students of deficiency disease In 1912, Holst and Frolich[718] published further studies on experimental scurvy. Similar alterations were described the same year in another species, the monkey, by Hart and Lessing[719] In both guinea pig and monkey the alterations produced by dietary means were identical with those which had been observed in infants at autopsy by a number of pathologists Hojer[720] studied scurvy in guinea pigs and reported his findings in 1924 During the next decade Wolbach[721 722 723] and his associates investigated the three main areas where scurvy manifests itself in the guinea pig connective tissue, bone, and teeth. Experimental ascorbic acid deficiency has been studied in monkeys, though tissue alterations have been given insufficient attention[1529] Wolbach's studies contributed much to our understanding of the dynamics of ascorbic acid deficiency, particularly with respect to reparative phenomena when the essential nutrient is restored to the depleted host We shall take up the three areas. bone, connective tissue and teeth in that order, drawing on the observations of Wolbach and on our own studies in experimental scorbutus

When a young actively growing (200-300 gram) guinea pig is placed on a scorbutogenic diet, weight gain continues for several weeks, then plateaus, and finally decreases. If the disease is allowed to run its course, the weight at autopsy will usually be below that present initially. Food intake by the animal decreases, to cease altogether towards the end Very little activity is displayed, nor will the guinea pig show its usual nervous reaction to a sharp sound. Deformity and swelling of the hind legs may be noted, and towards the end the animal appears to pull himself along by his front extremities The incisor teeth may become loosened. The fur becomes ruffled.

fact that ascorbic acid reduces silver nitrate; hence metallic silver is deposited at the site where the vitamin is present. Fine granules are prominent in the cells of the adrenal cortex, corpus luteum of the ovary, interstitial tissue of the testis and in the hypophysis.[708]

A number of miscellaneous functions have been ascribed to ascorbic acid, in the main these have been based on experimental observations in deficient guinea pigs. It is difficult to interpret and classify such observations so that they can be linked in any rational way with the pathological manifestations which will shortly be described. However, certain experimental findings are important and should be mentioned. One of the most interesting functions of ascorbic acid is its relation to the metabolism of the aromatic amino acids, phenylalanine and tyrosine. Ascorbic acid-deficient guinea pigs[709] and premature infants[710] excrete parahydroxyphenyllactic and parahydroxyphenylpyruvic acids in the urine when large amounts of phenylalanine and/or tyrosine are fed. Ordinarily such hydroxy-acids are completely metabolized and hence are not found in urine. The metabolic defect just mentioned is eliminated when adequate amounts of ascorbic acid are restored to the diet. *In vitro* studies with guinea pig tissue slices indicate that the main defect of vitamin C deficient tissues is an inability to oxidize the side chain of tyrosine rather than a failure to oxidize the benzene ring or conjugate the phenolic group.[711] The ascorbic acid-tyrosine relationship became even more interesting when folacin was found to prevent the hydroxyphenyluria produced in scorbutic guinea pigs when excess tyrosine was fed, even though it failed to protect against scurvy.[1055] This effect could not be demonstrated in monkeys[1057] or infants,[1056] (see page 272).

The relationship of ascorbic acid to adrenal cortical function is suggested by the high concentrations of the vitamin in the adrenal glands and by the enlargement of these structures in experimental scurvy. During the decade since the advent of adrenal cortical hormones much has been written on this subject which will not be detailed here Meiklejohn[712] has recently summed up the situation: "it seems fair to say that to date there is no undisputed evidence that ascorbic acid plays any part in assisting the synthesis or secretion of the adrenocortical hormone"

In scorbutic guinea pigs disturbances in carbohydrate metabolism have been described The significance of changes, such as decreased glycogen content of the liver, is not clear in experiments which may be complicated by the possible effect of inanition.[713]

Other metabolic defects have been observed in vitamin C-deficient animals. Decrease in the succinic dehydrogenase activity of heart and skeletal muscle has been reported,[714] as well as a rise in blood fibrinogen,[715] and a decrease in serum phosphatase activity.[716]

The outstanding investigations of Friedenwald and his associates[717]

which have been produced by naturally occurring trauma. In such a bone, the growth of the cartilage appears to be normal, cells continue to proliferate and increase in size. Blood vessels invade the cartilage cell columns in the usual fashion. The other main sequence in endochondral bone formation, the deposition of inorganic materials in the cartilage matrix between the hypertrophic cells, is also normal. But here the resemblance to the normal stops, for beneath the epiphyseal cartilage a broad and dense zone of calcified cartilage matrix is found. Vascular and myeloid elements as well as a few spindle shaped cells are present between the spicules of excess cartilage matrix. However, all is devoid of bone. From the appearance of such an immobilized, "artificially" scorbutic leg, the basic alteration is apparent: a complete cessation of osteoblastic activity, both formative and destructive. No bone matrix is found on the spicules of normally calcified cartilage matrix material, nor is there any evidence of removal of these.

If now we turn to the leg allowed to sustain the normal traumata of



FIGURE 65. ASCORBIC ACID DEFICIENCY.

Ribs, guinea pig. Adjacent ribs of guinea pig connected by intercostal muscles. Note differences in swelling of junctions, collapse, and marrow cells in relation to cartilage-shaft junctions. H and E. (x 75).

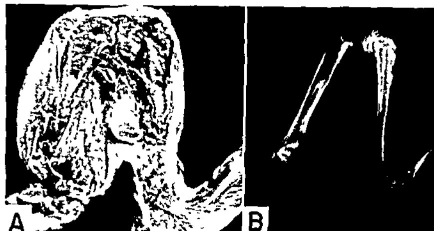


FIGURE 64. ASCORBIC ACID DEFICIENCY

Knee, guinea pig *A* Gross photograph of knee joint of guinea pig which had been allowed to die of scurvy. Note complete separation and dislocation of this upper tibial epiphysis on the shaft. *B* X-ray of same knee as in *A* Note separation of upper epiphysis which is seen in the gross photograph.

At autopsy very little is found grossly except for the changes in the skeleton. When the thorax is opened, the costochondral junctions are found to be enlarged, particularly those of the middle group of ribs. They may have completely separated If the knee joint is disarticulated, the epiphyseal cartilage of the upper tibia may be disengaged from the shaft. So, too, as the muscle is being removed from the lower leg preparatory to fixation, the periosteum may easily be detached from the diaphysis. These two phenomena, separation of both epiphyseal cartilage and periosteum from bone, epitomize the skeletal changes in experimental scurvy and, as we shall see later, in the naturally occurring disease in man as well.

When the bone is examined under the microscope, a specific and characteristic change is found, this is the classical textbook appearance of experimental scurvy described in the very beginning by Holst and Frolich [702] However, we should like to carry the development of the disease back earlier than this and to describe what might be called "synthetic scurvy." In order to effect this artificially-produced picture, it is necessary to protect the growing end of the bone from naturally occurring traumata such as those from weight bearing and muscle pull This may be accomplished by placing one extremity in a plaster cast before or during the early stages of the development of the deficient state.[724] The comparison of the leg in the cast with that of its unsupported fellow clearly shows that the changes in the latter are the result of an attempted effort at healing of the fractures

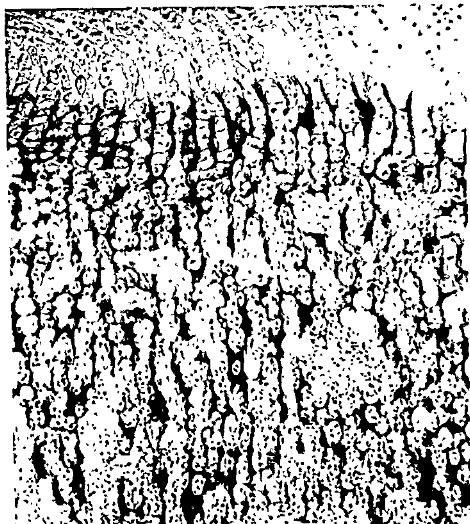


FIGURE 67. ASCORBIC ACID DEFICIENCY.

Upper tibia, guinea pig High power section shown in Figure 66A "Synthetic scurvy" This illustrates the prominent lattice with absence of osteoblasts and osteoid. Blood vessels are invading the cartilage which in turn appears to be growing in normal fashion The presence of the dense lattice indicates that its destruction is not taking place. H and E (x 200)

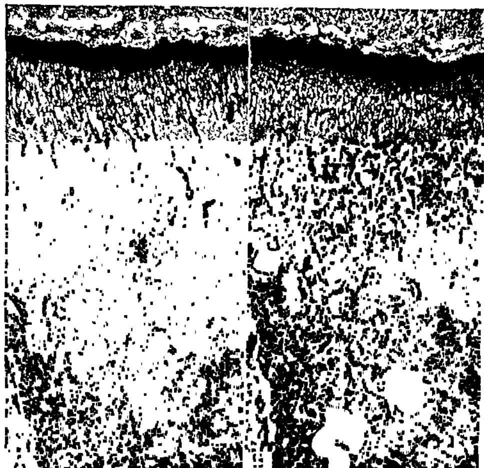


FIGURE 66 ASCORBIC ACID DEFICIENCY.

Upper tibia, guinea pig *A* From animal acutely deficient in vitamin C, to show disorganization, i e, presence of *Trummerfeld* and *Gerustmark* The zone of calcified cartilage matrix is perhaps a little broader than one might see in the human *B* Tibia of opposite leg which had been placed in a plaster cast to immobilize it at the beginning of the experiment. Notice prominent lattice which shows no fractures There is no migration of the marrow cells down into the shaft nor is there any evident proliferation of fibroblastic-like cells This illustrates that if the normal stresses and strains of muscle pull are eliminated classical evidence of scurvy with fractures, hemorrhages, et cetera, will not develop

do not deserve to be called such. Many red blood cells lie free in the ti: Erythroid and myeloid cells, that is red and white cell forming elem are conspicuously absent. Their disappearance gives the area below *Trummerfeld* a loose fibrous appearance, which in infants Schoed called the *Gerustmark*. About the fragments of calcified matrix, an eo philic homogenous material is found The bone of the shaft appears fied, making it inescapable that a certain amount of destruction is ta place in this region. Hemorrhages may be found beneath the perioste With the continued growth of the cartilage and the formation of more more calcified matrix, this area becomes weakened, hence complete sep tion of the shaft is to be expected. In the ribs or in the extremities a



FIGURE 69 ASCORBIC ACID DEFICIENCY.

Tibia, guinea pig A Field from Figure 68 to show fracture of a spicule of cal cartilage matrix Note relatively few cells in its vicinity These are spindle shaped T is no evidence of new bone matrix formation *B* Field from Figure 68 to show *Ge mark* which consists of a loose framework of spindle-shaped cells about a spicul bone in which a core of original calcified cartilage matrix remains No osteoblastic tivity is present nor are there any marrow cells H and E. (x 250).

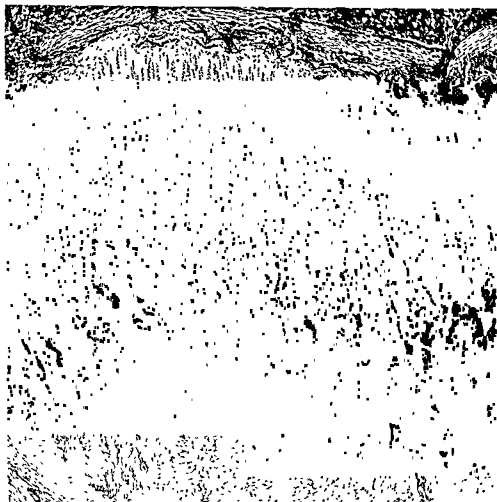


FIGURE 68 ASCORBIC ACID DEFICIENCY

Upper tibia, guinea pig This shows normal cartilage and broad lattice of excessive calcified cartilaginous matrix The under portion of this has fractured with a resulting *Trummerfeld* Note absence of marrow cells in this area Compare with Figure 55 which is from a normal guinea pig of the same age and at the same magnification H and E (x 60).

motion, muscle pull, etc., or to the ribs, constantly subjected to respiratory movements, the classical expression of scurvy is found Instead of orderly spicules of cartilage matrix, the picture is one of disorganization, a veritable area of debris, the *Trummerfeld* described by Fraenkel in infants.[725, 726] Spicules of matrix have broken off from the more recently formed material closer to the cartilage. The area is infiltrated with many spindle-shaped cells rather resembling fibroblasts, though, as we shall shortly see, they

do not deserve to be called such Many red blood cells lie free in the tissue. Erythroid and myeloid cells, that is red and white cell forming elements, are conspicuously absent. Their disappearance gives the area below the *Trummerfeld* a loose fibrous appearance, which in infants Schoedel[627] called the *Gerustmark* About the fragments of calcified matrix, an eosinophilic homogenous material is found. The bone of the shaft appears rarefied, making it inescapable that a certain amount of destruction is taking place in this region. Hemorrhages may be found beneath the periosteum. With the continued growth of the cartilage and the formation of more and more calcified matrix, this area becomes weakened, hence complete separation of the shaft is to be expected In the ribs or in the extremities a false



FIGURE 69. ASCORBIC ACID DEFICIENCY.

Tibia [illegible]



FIGURE 68 ASCORBIC ACID DEFICIENCY.

Upper tibia, guinea pig This shows normal cartilage and broad lattice of excessive calcified cartilaginous matrix The under portion of this has fractured with a resulting *Trummerfeld* Note absence of marrow cells in this area Compare with Figure 55 which is from a normal guinea pig of the same age and at the same magnification. H. and E (x 60).

motion, muscle pull, etc , or to the ribs, constantly subjected to respiratory movements, the classical expression of scurvy is found. Instead of orderly spicules of cartilage matrix, the picture is one of disorganization, a veritable area of debris, the *Trummerfeld* described by Fraenkel in infants [725, 726] Spicules of matrix have broken off from the more recently formed material closer to the cartilage The area is infiltrated with many spindle-shaped cells rather resembling fibroblasts, though, as we shall shortly see, they

cannot be demonstrated In the *Trummerfeldzone* no argyrophilic reticulum fibers can be made out.

Following specific therapy with ascorbic acid, striking reparative sequences ensue. The spindle-shaped cells round up, their cytoplasm increases in amount. This augmentation of basophilic cytoplasmic material appears to be the result of newly formed ribose nucleic acid, since its staining properties are destroyed following digestion with crystalline ribonuclease. Cytochrome oxidase activity reappears So, too, the presence of abundant alkaline phosphatase can be found twenty-four hours after therapy. In addition to these evidences of changed metabolism of the osteoblast, its altered activities are further exemplified by the appearance of a fine network of reticulum fibers, again as early as twenty-four hours after the initiation of therapy Such argyrophilic fibers coalesce to form broader bundles which no longer stain with silver, though the picture becomes obscured by the deposition of inorganic materials in this organic matrix or osteoid. The argyrophilic fibers are metachromatic and also stain positively with the periodic acid-leucofuchsin reagents. The possible significance of these

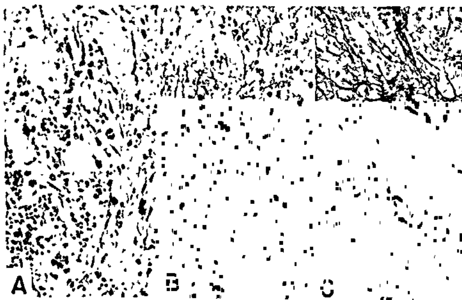


FIGURE 70 ASCORBIC ACID DEFICIENCY

Healing, rib, guinea pig *A* Cellular area from zone of *fractures to show absence of* any fibrillar material about cells This is the picture of absolute scurvy *B* One day after institution of therapy A few argyrophilic fibers have appeared *C* Five days after therapy. Fibers have coalesced into bone matrix Gomori silver stain all (x 195).

joint is produced, a sort of ball and socket formed from the convex cartilage and concave shaft. Naturally, secondary changes may be expected to occur in the cartilage as a result of motion.

Certain features of ascorbic acid deficiency, as it affects the bones, can be further explored by conventional histochemical techniques;[728] the effects of specific therapy on reparative processes, which Wolbach did so much to elucidate,[722] may also be investigated.

When the epiphyseal or costal cartilages from scorbutic animals are stained for glycogen, this material continues to be found in normal fashion, even in absolute scorbutus. However, some qualification must be inserted here, for, as the disease progresses, the animal ceases to eat, hence the effects of nonspecific inanition make themselves felt. These are exemplified by a reduction in the number of hypertrophic cells which result from a slowing in the growth of the cartilage as a whole. The matrix of the cartilage shows no reduction in metachromasia or change in its reaction with periodic acid-leucofuchsin However, due to what we have interpreted as mechanical effects, the epiphyseal or costal cartilage may become distorted and stretched laterally. Such spreading apart of the intercellular matrix reveals its fibrillary structure We have never been impressed with any specific effect of vitamin C deficiency on the cartilage. Such a statement is, unfortunately, in complete disagreement with Dr. Wolbach who, in one of his final papers, has written, "we are pleased to record the ease with which the cessation of cartilage matrix formation in ascorbic deficiency can be shown in epiphyseal cartilages of the long bones" and "matrix formation of epiphyseal cartilage cells ceases as a result of ascorbic acid deficiency just as promptly and conspicuously as does the matrix formation of bone and connective tissue cells and that cartilage cells do not mature until ascorbic acid is given." [729] We are in agreement with Dr. Wolbach as to the morphological changes and have described them ourselves. However, they are absent in the case of extremities which have been immobilized in a cast [724] Moreover, the promptness with which cartilage responds nonspecifically to the effects of inanition (page 13) makes us wish to keep this question open Perhaps forced feeding to maintain the intake of all nutrients save ascorbic acid would settle this matter once and for all

Another histochemical reaction, the demonstration of alkaline phosphatase activity, has been applied to cartilage in absolute scorbutus [728] No changes are noted save those related to the nonspecific effects of inanition

When the subepiphyseal or subcortical area of the shaft is examined histochemically, several abnormalities become evident. Cytochrome oxidase activity of the spindle-shaped connective tissue-like cells is absent or diminished; the cytoplasm of such cells is scanty. Alkaline phosphatase activity

several ways The chemist might define collagen as. "native fibrous proteins exhibiting specific spatial periodic wide angle x-ray diffraction patterns and containing 6 to 7 per cent of total N as hydroxyproline N, 10 to 12 per cent as proline N, 1 per cent as hydroxylysine N, and 25 per cent as glycine N".[724] The biophysicist might define collagen as· "a term embracing a class of fibrous proteins characterized by a particular wide angle x-ray diffraction pattern, many members of the class exhibiting a fiber axis period averaging 640 A. The known collagens of the higher animals are chemically characterized by a low content of aromatic amino acids and a high content



FIGURE 72 ASCORBIC ACID DEFICIENCY

A Skin, guinea pig Skin from back of normal animal in which an incision had been made nine days before in center Note absence of hair follicles and increased thickness of epithelium to which some debris is adherent. The wound is completely healed *B* Skin from a guinea pig suffering from acute scorbutus in which a similar incision had been made nine days before There is no attempt at healing whatsoever and, as will be seen, wide defect is present H and E. (× 6).

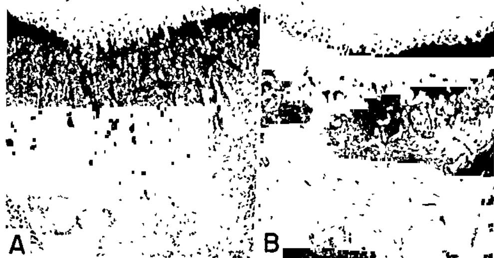


FIGURE 71. ASCORBIC ACID DEFICIENCY.

Healing, rib, guinea pig. Phosphatase reaction. *A* Section from animal dying of scurvy. Positive reaction is found in the cartilage and about the upper metaphysis Some of the blackening is due to inorganic materials. Note absence of reaction in cellular zone which is the object of showing this section so that it may be compared with *B* *B* After twenty-four hours treatment an intense reaction has appeared where none had been present before. Note also more activity in epiphyseal cartilage which has begun to grow (× 35).

changes in cartilage and bone, particularly following therapy, will be discussed below.

For historical reasons and because we ourselves are more familiar with skeletal alterations in ascorbic acid deficiency, the changes in the bones have been described first. In so doing the fundamental defect must have become apparent: the inability of certain cells to form those special supporting materials which are called connective tissue, osteoid, and dentin The fundamental characteristic of these tissues is that they are composed of a fibrous protein, collagen which is closely bound to one or more highly polymerized polysaccharides Although others, particularly Aschoff,[730] were cognizant of the basic change in scurvy, i.e, failure of certain cells to elaborate supporting tissues, Wolbach clearly elucidated in his experimental approach, particularly with respect to healing sequences, the fundamental alterations which occur in experimental scurvy in the guinea pig.[722]

Before detailing these changes, something should be said about collagen, which, depending on one's interests and training, can be looked upon in

scorbutic wounds, in contrast to large amounts in normal healing muscle lesions. Others have obtained equivocal results. The statement is made continually in the literature that Gersh and Catchpole [739] demonstrated the depolymerization of the ground substance in ascorbic acid-deficient guinea pigs These investigators were unable to furnish any chemical proof of depolymerization and only speculated that this might be the case Meyer,[749] too, has questioned the validity of Gersh's conclusions and has indicated that they cannot be correct since mucopolysaccharides are not stained by the histochemical reagents which have been employed. Today, since disturbances in the metabolism of mucopolysaccharides as evinced by vitamin reactions are used to explain so many diseased states, a critical appraisal by a chemist is refreshing and necessary

Our understanding of the dynamics of collagen formation and metabolism and the role of ascorbic acid thereto has increased considerably during the past few years The structure of collagen as visualized with the electron-microscope and the ways in which the collagen molecule can be reconstituted have served to stimulate much interest in this field In the organism collagen is a rather inert material, at least its turnover is not particularly high when measured with radioactive-labeled glycine and compared with the turnover rates of intracellular proteins.[750] As might be expected, the turnover of collagen in bone is higher than that in other tissues, such as skin, liver and tendon There appears to be little difference in the content of collagen in tissues from scorbutic guinea pigs as compared with normals [751]

Important studies dealing with the biosynthesis of collagen have recently been reported by Gould and Woessner [752] In brief, a study was made of healing skin wounds from normal and scorbutic guinea pigs with respect to the concentrations of proline, hydroxyproline, and glycine Withdrawal of ascorbic acid from the diet leads after seven days to definite impairment of hydroxyproline formation Ordinarily the hydroxyproline appears in greatest concentrations during the sixth to eighth days after the wound has been produced in animals given ascorbic acid from the time of production of the lesion In contrast animals on a scorbutogenic diet produce no hydroxyproline until vitamin C is given In twenty-four to forty-eight hours large amounts appear From these experiments it may be tentatively concluded that ascorbic acid may be necessary for the synthesis of hydroxyproline from proline and may be related, too, to the incorporation of proline, hydroxyproline and glycine into the collagen molecule

A number of investigators have studied the periarticular and intraarticular changes which are found in the chronic scorbutic state in the guinea pig. These observations have been reviewed by Cruchaud [738] The lesions are as one might expect from the alterations which have already been described

In many of the old descriptions of scurvy on land and sea the state-

of pyrolidine amino acids and glycine." [732] Collagen is thus unique chemically in its amino acid composition. Because of the presence and constancy of hydroxyproline, determination of this amino acid enables one to assay the amount of collagen present in a given specimen [431] The electron microscopic picture is also characteristic.[430]

As for the polysaccharides associated with various types of connective tissue, a number have been characterized: hyaluronic acid; chondroitinsulfuric acid, types A, B, and C; chondroitin; and keratosulfate.[733, 749]

The role of ascorbic acid in the formation of collagen and polysaccharides has been studied from several standpoints, valuable information having been obtained from investigations of healing sterile wounds, organizing blood clots and healing subcutaneous abscesses. In the former type of experiment the now classical studies of Wolbach and Howe [721] conclusively demonstrated that, when incisions were made in the skin or muscle of scorbutic animals, the lesions failed to heal. Microscopic examination of such wounds revealed that, although there was extensive fibroblastic proliferation in both scorbutic animals and controls, the cells in the former tended to remain immature looking. Most important of all, such cells did not appear to be able to promote the deposition of collagen in normal fashion From such observations it must be concluded that failure of collagen formation characterizes the scorbutic state. Further studies by Wolbach [722] and others [734, 735] have confirmed and amplified the initial observations, both in the guinea pig and in man. When wounds are produced in scorbutic animals, a pink-staining fluid-like material appears about immature proliferating connective tissue-like cells. This substance has been postulated to be a variety of materials, but the feeling of most students of scurvy, particularly Wolbach, is that this material represents an ineffectual attempt at collagen formation. In controlled studies of the recovery processes following absolute scorbutus, Wolbach failed to obtain any evidence that fibrin is a precursor of collagen.[722] Instead, the homogeneous, pinkish-staining material mentioned above, which does not take collagen or silver stains, becomes fibrillary after treatment with ascorbic acid. Such fibers are argyrophilic and following their appearance, collagen can also be stained. Wolbach took the view that reticulum and collagen are elaborated or secreted by connective tissue cells. Particularly important is the observation that following recovery from the scorbutic state collagen deposition is always found in the immediate vicinity of the fibroblast-like cells.

The "amorphous collagen" described by Wolbach,[722] which he thought was the precursor of collagen, has evoked a great deal of interest Studies of healing wounds in scorbutic animals have not yielded consistent results Penny and Balfour [736] found a decrease in the metachromatic material of

scorbutic wounds, in contrast to large amounts in normal healing muscle lesions Others have obtained equivocal results. The statement is made continually in the literature that Gersh and Catchpole [739] demonstrated the depolymerization of the ground substance in ascorbic acid-deficient guinea pigs. These investigators were unable to furnish any chemical proof of depolymerization and only speculated that this might be the case. Meyer,[749] too, has questioned the validity of Gersh's conclusions and has indicated that they cannot be correct since mucopolysaccharides are not stained by the histochemical reagents which have been employed Today, since disturbances in the metabolism of mucopolysaccharides as evinced by vitamin reactions are used to explain so many diseased states, a critical appraisal by a chemist is refreshing and necessary.

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In many of the old descriptions of scurvy on land and sea the state-

ment is made that wounds, already thought to be well-healed, broke down during the development of the scorbutic state. This situation has been pointedly studied in guinea pigs.[737] Laparotomy wounds were allowed to heal for six weeks in normal animals. Then the animals were placed on a scorbutogenic diet. By the fourth week extraordinary changes were observed: "fibroblastic proliferation, regression of connective tissue elements and hemorrhages." Hence it is concluded that ascorbic acid is needed for the maintenance of scar tissue for a period of many weeks. These studies need to be confirmed and extended.

In addition to the changes in fibroblasts and the absence of collagen formation, certain other differences in the capacity of wounds to heal in scorbutic and normal animals should be mentioned. The hemorrhage, which occurs as a result of the incision, is absorbed much more slowly and may never completely disappear in deficient guinea pigs. Then, too, although endothelial cells proliferate, capillary loops fail to invade the injured area (see below). These histological observations have been extended by others to obtain data on the ascorbic acid content and tensile strength of healing wounds in normal and scorbutic guinea pigs.[735] The vitamin C content of healing wounds of deficient animals is found, as expected, to be much lower than that of animals on an adequate intake of this nutrient. However, no change in concentration of ascorbic acid in the wound site of the deficient guinea pig over the concentrations in the skin elsewhere can be demonstrated. If air is injected into the peritoneal cavity and the pressure measured until the abdominal wound breaks down, the average pressure for wound rupture in scorbutic animals is found to be 127 mm. mercury, while in controls it is twice as great, or about 258 mm mercury.

The histological development of subcutaneous abscesses has been compared in scorbutic and normal guinea pigs; in such experiments the paired-feeding technique was utilized.[740] The animals were innoculated with a strain of hemolytic staphylococcus aureus. Microscopically, in the first few days there was a prompt outpouring of polymorphonuclear leukocytes in both groups of animals; phagocytosis appeared to be active. By the third day, there tended to be fewer macrophages in and about the lesion of the deficient animals in contrast to large numbers in the controls There was also little connective tissue proliferation in the former group. A week following innoculation the lesion was localized in the deficient animal, but a wide zone of connective tissue cells was found about the necrotic focus; between the cells was a pinkish-staining material together with numerous red blood cells. No capillaries were found growing into the center of the lesion; instead "defective looking" dilated vessels were found at the periphery. A week later the zone about the abscess was even wider in the deficient animal, in contrast to the compact well-encapsulated lesion

of the control From this study it was concluded that although no decrease in polymorphonuclear leukocyte response occurs, the macrophage reaction is delayed and is less than that encountered in the controls. Phagocytosis by the mononuclears also appears abnormal. As was expected, there is also an inability of the scorbutic animal to produce collagen and organize his abscess Grossly, therefore, the lesion in the deficient animals is diffuse and soft in comparison to that in the controls which is rounded up and firm It is interesting to speculate on the role of blood vessels in relation to the lesion and its pathogenesis. Whether defective capillary invasion retards the influx of macrophages is difficult to determine. It seems unlikely, however, that poor circulation has any effect on poor collagen formation.

Histological studies of dental and peridontal structures have been reported in scorbutic guinea pigs in which the changes may be extensive, probably because the growth of the incisor is so rapid Two millimeters are erupted on an average each week in comparison to only a few millimeters a year in the human.[741]

As might be expected, the most marked alterations in the teeth are found in the dentine. Wolbach and his associates[723, 742, 743, 744] have extensively studied the pathogenesis of the changes When guinea pigs are placed on a scorbutic diet, alterations very soon appear in the odontoblasts, these cells become atrophic so as to resemble the nearby pulp cells They lose their orderly polar arrangement, becoming completely disorganized The vessels of the pulp dilate and red blood cells appear to ooze through. As a result of these changes in the odontoblasts, dentine is laid down irregularly, thus the dental tubules appear to be arranged in a haphazard fashion After a time, dentine deposition stops entirely. The predentine becomes hypercalcified A few of the odontoblasts in the pulp apparently form some dentine, at least enough to enclose themselves The alizarin technique has been employed to demonstrate that dentine formation is quantitatively related to ascorbic acid intake[743]

Changes in the enamel organ occur later in the course of the deficiency The ameloblasts atrophy and hemorrhages are encountered. Both these alterations have been interpreted to result from traumatic injury of the enamel organ since its support is poor No evidence of any relationship between ascorbic acid deficiency and dental caries has been presented Rarefication of the alveolar bone is present, as might be expected, when one recalls the changes encountered in the ribs and other bones of experimental animals Weakness of the supporting bone and collagen fibers allows for greater mobility, consequently, there is decreased ability to withstand the mechanical stresses encountered in chewing The changes in the supporting structures of the guinea pig have been likened to the diffuse alveolar bone atrophy of pyorrhea encountered in the human.[742]



FIGURE 73 ASCORBIC ACID DEFICIENCY.

Teeth, guinea pig. *A* Mandible of guinea pig cutting the first molar and the lower incisor transversely. Acute scurvy. There is complete cessation of dentin formed before the deficiency became complete *B*. Chronic scurvy. This shows imperfect osteo-dentin formation. The peridontal membrane is approximately twice the normal width (Courtesy of Dr Paul E. Boyle.)

Hemorrhages are a prominent feature of experimental ascorbic acid deficiency and are found *par excellence* at sites where naturally occurring trauma is apt to manifest itself, i.e., the joints, ribs, diaphragm, et cetera. The cause of the extravasation of blood has usually been ascribed to capillary weakness, perhaps a defect in the spaces between endothelial cells as a result of a lack of "intercellular cement substance." When the mesenteric vessels in scorbutic guinea pigs were pointedly studied by Lee and Lee,[745] two important functional alterations were found as compared with normal animals. First, a decreased responsiveness of certain vascular elements to physiological concentrations of epinephrine was observed. Such a change was most marked in the prearterial portion of the capillaries. Secondly, there was a distinct tendency for the terminal collecting vessels draining the capillary bed to become dilated and engorged and thus more easily liable to traumatic rupture. Further studies[746] have shown that scorbutic guinea pigs respond to severe hemorrhage by failing to produce renal vaso-humoral agents (VEM) as do normals; moreover, the kidneys of ascorbic acid-deficient animals fail to produce VEM *in vitro*.[747] It would appear that these functional circulatory changes are more important than the anatomical alterations which have been conjectured as a result of the hemorrhages rather than specifically demonstrated. That there is no increased generalized vascular permeability in vitamin C-deficient guinea pigs can be shown by injecting a dye such as T-1824.[748]

Wolbach maintained that endothelial proliferation was reduced or even absent in experimental scurvy. He based his conclusions on the lack of organization of experimentally-produced blood clots, that is, a failure of connective tissue cells and blood vessels to grow into such areas. There is an alternative explanation, however. Could it be that the capillary endothelial cells need a connective tissue framework upon which to grow? From our own studies in the guinea pig, we have seen no reduction in the invasion of epiphyseal cartilage by newly-forming blood vessels. Such endothelial structures had the framework of the calcified cartilagenous lattice upon which to grow. This problem requires further investigation.

Ascorbic Acid Deficiency in Man: Deliberate attempts to produce ascorbic acid deficiency in infants have been few. Most instances of scurvy at this age occur from failure to include a source of vitamin C in the diet (page 398). In certain of the premature babies studied by Levine *et al*,[710] ascorbic acid was withheld from the feeding formulas in order to measure urinary hydroxyphenyl acids. Abnormal concentrations of these acids were found, which disappeared following appropriate therapy.

Much more information on the pathogenesis of scurvy in the human has come from studies of experimental scurvy in adults of which there have been several reports. Crandon's experiment upon himself[1530] furnishes ex-

tremely interesting data on the biochemical and pathological changes which occur as the scorbutic state develops. Crandon's plasma ascorbic acid level fell to zero after forty-one days on a diet consisting of bread, meat, corn flakes, cake and butter. The white cell-platelet ascorbic acid concentration fell to zero after about the twelfth week of the deficiency. No other significant findings were present until 134 days had elapsed. At this time small perifollicular hyperkeratotic papules began to appear. Such lesions resembled those which previously had been described as characteristic of vitamin A deficiency (page 359). After three months, a wound was made in the skin and subcutaneous tissues; this appeared to heal in normal fashion Examination of microscopic sections corroborated the gross interpretation At the end of 182 days, after the white cell-platelet ascorbic acid had been at zero for sixty-one days, histological examination of a second wound showed virtually no healing. Since this furnished evidence that scurvy based on morphological criteria was present, the experiment was terminated Because so much has been written of the role of ascorbic acid in the etiology of gingivitis in the human, it is of interest that no lesions appeared in Crandon's gums. At the present time, based on experimental studies and other observations, the feeling is that, few if any cases of gingivitis and bleeding gums result from ascorbic acid deficiency when oral hygiene is maintained.[1531] In view of the changes of the guinea pig teeth referred to above, it is of interest that one of the abnormal manifestations which appeared in the human experiment was interruption in the lamina dura, which doubtless results from atrophy of the alveolar bone. Crandon did not develop anemia.

The most exhaustive study of vitamin C deprivation in man has been reported from the Sorby Research Institute at Sheffield, in England.[1532] Twenty volunteers made up the group, of which ten were on the ascorbic acid-deficient regimen for as long as sixty weeks. The first clear-cut evidence of clinical scurvy was seen after about seventeen to twenty-four weeks, the change which was observed in all consisted of enlargement and keratosis of the hair follicles. In time such areas became hyperemic and later hemorrhagic and thus gave rise to characteristic scorbutic spots The vascular changes were carefully examined with the microscope. The initial alteration which was observed after the hyperkeratotic lesions made their appearance consisted of the formation of new capillaries; these increased in number, as might be expected from the nature of the increasing vascularity seen grossly. Next red cells were observed outside the capillaries. These were then seen to disintegrate giving rise to localized deposits of brown pigment. With therapy the capillaries and pigment disappeared.

The next clinical manifestation was in the mouth, where changes were seen in the gums after about six months. The initial alteration was slight

erythema and swelling of the tips of the interdental papillae. Small hemorrhages next appeared and as time went on the remainder of the gums became reddened, swollen, and finally loosened and "sagged away" from the teeth. The color then changed from a bright red to purplish hue and the gums soon became ulcerated Repair was slow following therapy, though eventually complete restitution was seen

Another prominent finding was the exacerbation of acniform lesions in several individuals who were suffering from this disease at the start of the experimental period Almost all the deficient group complained of pain in the back, joints, and extremities as time went on Effusions into the knee joints were seen in one of the volunteers

Incisions were made to study wound healing sequences and then were removed In the scars produced by this procedure hemorrhages were seen at the height of clinical scurvy

A number of laboratory procedures were carried out but indicated no alterations from the normal These included hematological studies and determinations of plasma protein, serum urea, and serum alkaline phosphatase concentrations.

To conclude this discussion of the effects of ascorbic acid deficiency it is important to point out that ascorbic acid deficiency and scurvy are not necessarily synonymous terms The term, scurvy, should be restricted to the clinical and pathological changes first described in adults (page 385) and later in infants (page 387). In the former group the conspicuous features are bleeding gums with loss of teeth, perifollicular cutaneous hemorrhages, and weakness of the lower extremities In infants the disease is characterized by subperiosteal hemorrhages, separation of the epiphyses, swollen gums and cutaneous hemorrhages The other manifestations of ascorbic acid deficiency, which have been noted above, should not be included in the classical scurvy syndromes of adults or infants

THIAMINE

Modern knowledge[755] of thiamine began in the 1890's with the classical studies of Eijkman, who demonstrated the nutritive value of rice polishings in pigeons which had been fed a diet of polished rice. In 1911, Funk isolated a crystalline fraction with biological activity from rice polishings During the following quarter of a century, extracts of increasing potency were prepared for use in the treatment of beriberi in the human. In 1926, Jansen and Donath obtained a pure crystalline material. In 1936, Williams and his associates[1480] were able to announce the structural formula and synthesis of a pure, biologically active substance, which, since it contained sulfur, was named thiamine.

During the years preceding the synthesis of thiamine, a coenzyme called cocarboxylase, which had been isolated from yeast, was extensively studied In 1937, when Lohmann and Schuster showed that this material was the pyrophosphoric ester of thiamine, the biological role of thiamine became apparent Thiamine pyrophosphate (cocarboxylase) is made up of pyrimidine and thiazole rings plus phosphoric acid.

Ingested thiamine is phosphorylated in large part by the liver and to a lesser extent by the kidney Very little free thiamine occurs in tissues, almost all is found as the pyrosphosphate[756] The organism excretes thiamine in its phosphorylated form

In 1936, Peters described the "biochemical lesion" of thiamine deficiency When the vitamin was added to suspensions of brain tissue from thiamine-deficient pigeons, the pyruvate content of the mixture was reduced.[757] Since these now classical studies, knowledge of the relationship of thiamine to carbohydrate metabolism has been greatly broadened, this vitamin appears to participate in all oxidative decarboxylations which lead to the formation of CO_2 Moreover, it affects other reactions such as, oxidation, dismutation, and condensation.[758]

Experimental thiamine deficiency leads to physiological and anatomical alterations in rats,[773, 782] mice,[802] hamsters,[763] cotton rats,[876] cats,[771] dogs,[769] foxes,[774] swine,[772] sheep,[801] calves,[800] and monkeys.[759, 775, 790] Purified diets have not been used in the experiments on all of these species, however. Poor food consumption may be partially responsible for any growth disturbance which may be observed because of the anorexia which usually accompanies the thiamine-deficient state Hence, inanition control animals must always be included in experiments which study thiamine deficiency.

Tissue concentrations of thiamine are reduced when the dietary intake of this vitamin is restricted.[756] As a result metabolic disturbances may be

observed in the thiamine-deficient organism. Elevations of blood pyruvic acid have been found in most species. For instance, pyruvate levels as high as 9.9 mg per cent have been observed in the blood of monkeys; the average normal value is 3 2 per cent.[759] Accompanying such alterations in tissue metabolites, a derangement of respiration has been observed in cardiac and skeletal muscle, as well as in brain, kidney, and liver. Of particular interest have been *in vitro* studies of the Q_{O_2} of heart muscle of rats,[760] in view of the histological changes shortly to be described. Although the oxygen consumptions of the ventricles of thiamine-deficient and normal animals are about the same, those of the auricles from the former group are significantly lower; the ratio of the oxygen consumptions of auricle to ventricle is 1.4 for thiamine-deficient animals and 2.0 for normal controls. *In vitro* studies of myocardium from thiamine-deficient rats reveal a decreased ability to metabolize pyruvate.[761] This derangement is related to a reduction of the tissue concentrations of thiamine. *In vivo* studies in dogs have shown that the myocardium of thiamine-deficient animals can remove less pyruvate than can the normals.[762] It will be recalled that the myocardium is dependent for its energy on carbohydrate, particularly pyruvate. In this it differs from skeletal muscle. As will be seen below, the heart muscle fibers, not those from voluntary muscle, suffer most severely as a result of thiamine deficiency.

Several analogs of thiamine are known and have been utilized to produce thiamine deficiency states [754] For instance, when oxythiamine is given to rats an increased pyruvate concentration is found in the blood together with an increase in the excretion of thiamine. Another interesting phase of thiamine metabolism is related to the presence in certain fish and shell fish tissues of an active enzyme, thiaminase, which splits the vitamin.[764] So, too, the possibility that the intestinal flora might contribute to the destruction of the vitamins must also be considered. This matter has been studied by Japanese workers [765]

In those species in which detailed physiological or anatomical observations have been carried out, fairly consistent changes have been found in the heart as a result of thiamine deficiency. Some time ago bradycardia was described as a distinctive feature of vitamin B_1 deficiency in the rat.[766] This observation has been confirmed by other investigators in rats,[767, 768] dogs,[769] swine,[770] cats,[771] and monkeys.[759] The possibility that inanition may lead to bradycardia must always be considered, as has already been noted. In swine which we studied in association with Wintrobe *et al*,[770] thiamine deficiency appeared to produce a *greater degree of slowing of the heart rate* than that which could be ascribed to inanition alone. Vagal overactivity may be a cause of the bradycardia, since slowing of the heart was observed in one animal to which atropine was administered. Further evidence of dam-

age to the myocardium has been furnished by electrocardiographic studies which have been reported in rats,[767, 768] dogs,[769] cats,[771] swine,[770] and monkeys.[759] For instance, extensive, though nonspecific, alterations, which have been described in swine, consist of prolonged P-R intervals, abnormalities in the P wave, increase of T_4, nodal and ventricular premature beats, A-V dissociation, complete block, and auricular fibrillation.[770] The tachycardia which has sometimes been observed in experimental animals has been interpreted to be an expression of cardiac decompensation

Objective evidence of cardiac failure has appeared prominently in swine,[772] especially those animals having a severe, acute thiamine deficiency. Such pigs exhibit labored breathing and cyanosis, both of which are made worse by exercise. A number of animals have died suddenly; no other cause for death save heart failure has been found after careful histological examination of the heart and other tissues.

At autopsy, the heart of the thiamine-deficient animal is usually described as dilated (rats, dogs, swine) Evidence for cardiac hypertrophy is extremely difficult to evaluate. Abnormally high heart weight-body weight ratios have been found in a few of the swine we have observed, it must be

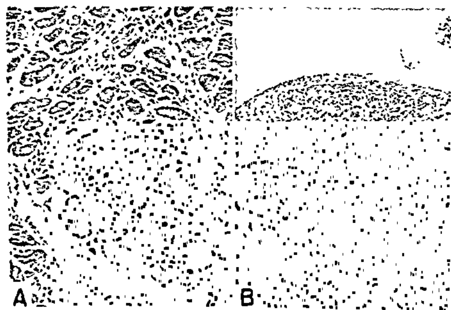


FIGURE 74 THIAMINE DEFICIENCY.

[illegible]

from animal which died suddenly after thirty-seven days of the deficiency (x 75).

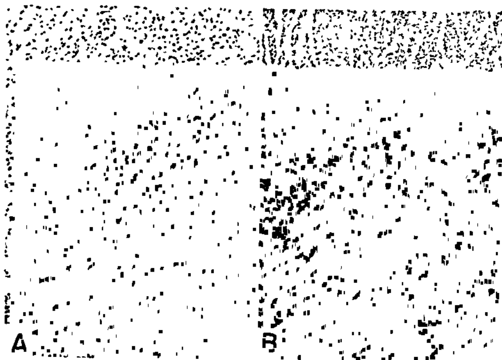


FIGURE 75. THIAMINE DEFICIENCY.

Heart, pig *A* Small focus of necrotic fibers infiltrated by leukocytes from an animal which had been on a thiamine-low regimen for 156 days (x 90). *B* More diffuse change with extensive cellular reaction (x 35). This animal died in clinical heart failure

emphasized, however, that in other animals whose growth may be greatly retarded by various means, the heart-body weight ratio may also be increased; for instance, in other deficient states, the heart may approximate 1 0 per cent of the total weight, which is the highest ratio to be observed in thiamine-deficient swine.

Microscopic lesions in the myocardium have been described in rats,[773] dogs,[769] foxes,[774] swine,[772] and monkeys.[775] The most extensive alterations with which we are familiar have been found in the myocardium of swine where changes may appear as early as the thirty-seventh day of the deficient state. Initially one finds a loss of striations accompanied by vacuolation and hyalinization of the myofibrils This is followed by total necrosis Leukocytes, both polymorphonuclear and mononuclear, appear. The necroses may be focal or diffuse, in one animal which we observed, the lesions could be seen grossly. Foci of dead fibers are found in the myocardium of both auricles and ventricles. An exception has been noted in one pig dying at an early stage, in this animal only the auricular musculature was affected.

ıficant dıfference in the response of the auricular and ventricular lature to thıamıne deficıency is further suggested by observatıons ın hose auricles are found to be ınvolved far more frequently than are ıtricles.[773] In thıs connection ıs it ınterestıng to recall the dıfferences oxygen consumptıon of auricular and ventrıcular muscle from thıa-leficient rats [760]

swıne whıch have passed through several epısodes of clınıcal thıamıne ncy scars may be found ın the myocardıum at autopsy These have nterpreted to ındıcate focı of prevıously necrotıc myocardıal fibers. oronary vessels of thıamıne-deficıent anımals, as well as the endo-m and epıcardıum, are not remarkable, no mural thrombı have been ed ın swıne or other specıes A poınted study of the conductıon sys-as not been made

e cause of the bradycardıa, electrocardıographıc changes and morpho-. lesıons ıs obscure Since the accumulatıon of certaın metabolıc

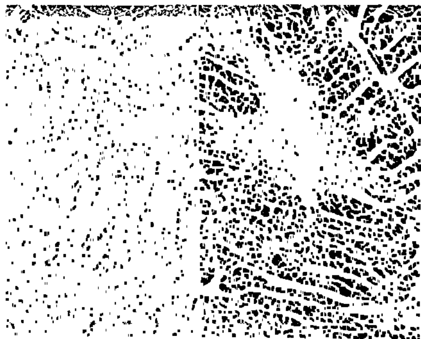


FIGURE 76 THIAMINE DEFICIENCY.

pıg A Fıne scarrıng between ıntact muscle fibers ın ventrıcle of anımal whıch een on thıamıne-low dıet for 320 days There ıs relatıvely lıttle ınflammatory, hough ın other areas acute lesıons were present (x 75) B Large scar ın myo-m of anımal whıch had been on thıamıne-low dıet for 254 days and whıch d chronıc passıve congestıon (x 60)

products may be responsible, large amounts of pyruvic acid, sodium pyruvate, and related substances have been administered to normal and thiamine-deficient rats.[776, 777] Under such circumstances only slight changes are observed in the heart rate and electrocardiogram. Since a sustained elevation of blood pyruvic acid has not been artificially produced, it is not clear whether accumulations of such metabolites are an important factor or not. We are, therefore, at a loss to explain the changes specifically, except to refer them to the metabolic defects which are known to accompany thiamine deficiency.

It is of interest to recall that cardiac lesions identical with those produced by thiamine deficiency have been observed in animals deficient in potassium.[70] When these two deficiencies are simultaneously produced no lesions whatsoever appear in the myocardium.[77] The reason for this requires further investigation.

No anatomical alterations have been observed in the striated muscles of thiamine-deficient animals, necroses have been described in rats in which a concurrent deficiency in potassium and thiamine has been produced [77]

Thiamine deficiency has been indicted as the cause of lesions in the nervous tissues Here the pathological alterations are not as clear-cut as are the changes which have just been described in the heart.

The classical studies reported in 1897 by Eijkman[778] were carried out on fowl which had been placed on a ration of polished rice. The syndrome was described as follows: "The beginning of the disease is characterized by an unsteady gait which first of all manifests itself in walking about on the perch, as if the animal cannot squeeze its toes around it firmly enough, and must exert itself in order not to fall off. The disturbance in motility soon increases in intensity and speed. The fowl no longer has the strength to climb up; because of weakness it holds its limbs spread apart and bent at the knee and ankle joints; when running it frequently collapses or falls over. Finally, it remains lying on its side and in its fruitless efforts the developing paralysis of the wing muscles also becomes noticeable The paralysis of the body musculature rapidly progresses from below upward."

Eijkman later epitomized the pathological changes:[779] "The involvement of the peripheral nerves is the most important feature that post-mortem investigation reveals to date. It involves both the sensory and motor portions, which occur focally in the nerve trunks, and produces the picture of non-inflammatory atrophic degeneration such as is observed after transsection of a nerve in the distal portion of the divided fragment. However, definite changes in the spinal cord and spinal cord roots are also not lacking. These show, likewise, the appearance of degeneration and atrophy."

On the basis of such findings Eijkman referred to the experimental disease as "polyneuritis gallinarum" Eijkman's experiments were repeated by

Vedder and Clark[780] who have illustrated degenerative lesions in the peripheral nerves.

It should be clearly understood that the diets employed by Eijkman and Vedder were composed of polished rice. Such diets are inadequate in many respects as McCollum[23] pointed out many years ago; for besides being deficient in minerals, particularly calcium and sodium, polished rice lacks most of the vitamins, and furnishes a poor quality protein. "Polyneuritis gallinarum" as studied by the early workers is not a syndrome due to lack of a single nutrient but clearly one caused by deficiency of several.

From these studies in birds it was concluded that the purified material from rice polishings was an antineuritic vitamin. When a characteristic syndrome, which could be prevented by extracts of rice polishings, was also observed in deficient rats, the term "polyneuritis" was applied to the condition in that species as well. Such rats display lameness of the fore and hind legs; they walk unsteadily with the extremities extended. Ataxia may be present, accompanied by cartwheel or rolling movements, convulsions have been observed. *Cure of this syndrome in rats as a test for vitamin B_1 was* first introduced by McCollum and Simmonds in 1918[782] and has been used by many subsequent investigators,[783] most of whom seem to have had little doubt that they were dealing with animals in which morphological changes in the nervous tissues were present.

The following discussion of the neurological aspects of thiamine deficiency may be separated into two parts: a consideration of the peripheral nerves and an appraisal of the central nervous system. In evaluating the experiments which are to be cited below, several facts must always be borne in mind. First off, autoclaved yeast has been used as a source of the B group in many of the experiments, since heat destroys thiamine. It is well to remember that excessive temperatures may destroy other components of the B complex, for instance pantothenic acid.[789] Secondly, animals on a thiamine-deficient diet fail to eat, consequently the effects of inanition must always be rigidly controlled. Finally, many of the diets have not contained all of the now recognized essential nutrients, particularly vitamin K, and in birds, certain amino acids.

The observations on birds, which had been placed on rice diets, profoundly influenced pathologic investigations in other species until careful studies were carried out in the latter group. Examination of the peripheral nerves of rats fed diets containing autoclaved yeast has revealed no significant morphological differences from control animals.[784, 785, 786, 787] The changes which do appear may be ascribed to inanition. The behavior of the animals may well be ascribed to "biochemical lesions" without anatomical changes. After cats have been eating a thiamine-deficient diet for as long as 116 days, no histological changes can be detected in the peripheral nerves

In addition, more conclusive evidence is furnished by studies of nerve action potentials of such thiamine-deficient cats and their normal controls. No differences are found, nor is there any disturbance in the regenerative capacity of the peripheral nerves of the thiamine-deficient animals [788] In association with Wintrobe *et al*, we have failed to find any evidence that thiamine deficiency leads to morphological changes in the peripheral nervous system of swine [789] Nor have lesions been found in monkeys.[790]

In contrast to the mammalian species just mentioned in which evidence of myelin degeneration of the peripheral nerves has been found, data have been presented, chiefly by Swank and his collaborators,[791, 792] which indicate that in pigeons lesions do occur. Heretofore, Aves have not been considered in this book. Since the observations of Swank are at variance with those encountered in all mammalian species so far studied, it seems desirable to mention them, inasmuch as they have continued to enjoy a good deal of prominence in contemporary nutrition. When young pigeons are forced-fed diets containing very small amounts of thiamine, lesions consisting of myelin and axon degeneration of the peripheral nerves are observed. In assuming that food placed in the pigeon's crop is utilized, Swank has been criticized by Shaw and Phillips [793] who feel that inanition may have led to Swank's findings since "the natural tendency of the bird to reduce its caloric intake during the thiamine deficiency could not be overcome by introducing food into the upper part of the digestive tract." The experiments of Shaw and Phillips lend support to the view that chronic thiamine deficiency may play a role in the development of neurological lesions in birds. They are careful to point out, however, that other factors may be important, such as the amino acids, glycine and arginine, since the chick requirements for these nutrients are different from those of Mammalia. In concluding this discussion of the peripheral nervous system, there appears to be no good evidence that uncomplicated thiamine deficiency leads to structural or functional lesions of the peripheral nerves of any of the Mammalia thus far studied. The question in birds requires further investigation. A discussion of the situation in man will be reserved until later (page 405)

Changes which have been described in the central nervous systems of thiamine-deficient animals may be considered first from the physiological and then from the anatomical standpoint When rats are placed on a diet, the B vitamin supplement of which is autoclaved yeast, a significant disturbance of vestibular function appears, this is evinced by an increased duration of nystagmus following rotation.[794] Physiological alterations have also been demonstrated in thiamine-deficient cats whose diets were adequate in all other essential nutrients, including pyridoxine and pantothenic acid.[795] The course of the feline syndrome can be divided into three stages. The first, which lasts three to four weeks, is marked by increasing anorexia

and vomiting, ataxia is sometimes observed at this time The second stage is manifested by abnormal posture, ataxia, dilatation of the pupils, and the presence of abnormal reflexes, such as body righting, vestibulo-ocular and pupillary light reactions. The flexor, knee kick, and hopping responses are all normal This stage is followed by one in which convulsions are prominent, these precede death From the neurological signs which such cats exhibit, it has been concluded that the mid-brain is most severely involved, it is unfortunate that histological studies have not been carried out to confirm or deny such a supposition In another study in cats,[795] physiologic changes as severe as those just described have not been encountered; only ataxia and mild vestibular disturbances were observed. In this experiment it was thought that the animals succumbed as a result of cardiac damage. Ptosis, incoordination, and ataxia have been described in two separate groups of monkeys.[759, 790 796]

Morphologic lesions have been described in the central nervous tissues of rats, mice, cats, foxes, and monkeys In rats,[784] hemorrhagic foci, as well as chromotolysis or clumping of the Nissl substance of the nerve cells, have been noted in Deiter's nuclei, the vestibular nuclei, the nuclei of Bechterew,

said to be present in the vestibular nuclei and there is swelling of oligodendrocytes The course of such animals with respect to weight gain or loss and the absence of control observations make this study of questionable value, however

Thiamine has a marked curative effect on a spontaneous paralytic disease of foxes[774] The syndrome which was first reported from the Chastek fur farm in Minnesota is characterized by a rapidly progressing paralysis At autopsy, bilateral symmetrical, degenerative lesions of certain nuclear masses in the paraventricular regions are encountered. It has been concluded that this Chastek paralysis is the pathologic counterpart of Wernicke's hemorrhagic encephalitis in man, a point which will be discussed in more detail shortly. The disease in foxes results from the presence in raw fish of a factor which appears to be a thiamine destroying enzyme[764] When thiamine is administered to affected animals, recovery ensues. Despite this, deficiencies of other essential nutrients may also be present, since at autopsy on animals dying with Chastek paralysis, a severe degree of hepatic lipoidosis is observed. A similar disease occurring in cats fed commercial cat food has been described[781] Symptoms and lesions are prevented by thiamine administration

After monkeys have been placed on a thiamine-deficient regimen for several weeks, they cease gaining[796] The weight curve then plateaus or

begins to fall. Food consumption decreases with a coincident fall in the blood thiamine concentration. With the decrease in weight, the animals become inactive, apathetic, and weak. If treatment is not instituted, ataxia appears and is accompanied by ptosis and tremors. Retching may be observed but no outright vomiting. Convulsive movements may occur. The animal then goes on to develop paralysis, shortly followed by death. Edema is not prominent.

Cardiac changes in the monkey have been mentioned above. Of great interest are the cerebral alterations which have been found.[790] The lesions are focal and bilaterally symmetrical The most frequent areas of the brain which are involved are the caudate nucleus, putamen, globus pallidus, thalamus and substantia nigra. Other loci, including the gray matter of the spinal cord, are affected to a lesser extent. The focal, circumscribed areas of involvement are characterized first by an accumulation of fluid, which spreads the nervous elements apart. Next, one finds loss of myelin and necrosis of neurons. Glial involvement is evidenced by phagocytes and slight astrocytic proliferation. Vascular alterations are not prominent and are thought, when present, to represent a reparative process. In this respect the lesions in the monkey are different from those of the Wernicke syndrome in man, which have been ascribed to thiamine deficiency (page 415).

Again it is necessary to mention certain changes which have been described in pigeons by Swank *et al.*,[791, 792] and by Alexander and his group.[797] The latter fed pigeons a ration of rice, supplemented with riboflavin and vitamins A, C, and D. Since, on this diet, which is obviously inadequate in many essential nutrients, the birds develop hemorrhagic vascular lesions in the brain, Alexander has postulated without any justification whatsoever, that thiamine possesses "angiodegenerative properties." Swank describes similar vascular lesions in pigeons, as well as changes in nerve cells and fibers, particularly those of the vestibular system and the oculomotor group [798] The latter experiments must be questioned for the same reasons that Shaw and Phillips [793] pointed out and which were discussed above. Swank has also applied the technique of electroencephalography to supplement his morphological investigations.[799] During the initial stage of the deficiency the amplitude of the brain waves increases, later there is a reduction in frequency with occasional paroxysmal discharges of epileptiform-like character.

From the above we may conclude that, in contrast to the absence of lesions in the peripheral nerves of thiamine-deficient mammals, the brain shows well-marked changes, which appear to be responsible for at least some of the physiological disturbances which may be observed during life.

In *man*, thiamine deficiency has been produced under experimental conditions in order to elucidate certain components of the beriberi syn-

drome (page 405), i.e., the heart and the peripheral and central nervous systems.

Electrocardiographic alterations are a prominent manifestation of beriberi and such changes may revert to normal when thiamine is administered.[1356] Relatively insignificant changes in the electrocardiogram have been observed in experimental thiamine deficiency in man.[803] Clinically, no outspoken evidence of cardiac embarrassment has been detected although it is obvious that it would be hazardous to carry the thiamine-deficient state too far in view of its known deleterious effect on the heart of the experimental animal.

Although clinical "polyneuritis" has been described in experimental thiamine deficiency in the human, the data are not too convincing For instance, a purified diet consisting of casein, fat, sugar, salt, and vitamin supplements was employed to study the effects of thiamine deficiency on a series of individuals for as long as eighteen months.[803] The thiamine content of this diet was gradually reduced to zero Symptoms and signs appeared in four out of nine subjects and consisted of "neuritis" (otherwise unspecified), edema, anorexia, and sometimes vomiting. In another study two individuals were placed on a regimen in which there was 1 mg of thiamine per 1000 calories[804] This ration led to weakness, anorexia, and vomiting. In addition, evidence of neuro-muscular involvement appeared: numbness and tingling of the legs, sensory disturbances, tenderness of the calf muscles, weakness of the extremities, and loss of the achilles and patellar reflexes. It is extremely unfortunate that biopsies of nerve and muscle were not performed on these two subjects to confirm or deny the appearance of anatomical changes, especially since fifty days of thiamine therapy were required to correct the defects in one case, while the reflexes of the other subject did not respond even after four months of treatment The observations on these two subjects are the sum total of our knowledge concerning the relationship of thiamine to the integrity of the peripheral nervous system in the experimental human subject. Further discussion on the neurological component of the beriberi syndrome will be found on page 408.

RIBOFLAVIN

The biological importance of certain colored materials from various sources became apparent in 1932 when Warburg and Christian [805] announced the isolation of a yellow respiratory enzyme and showed that it could be split into two portions protein and pigment Shortly thereafter, several laboratories reported the isolation of yellow-green fluorescent pigments from a variety of sources. Among this group, Kuhn and his associates [806] described a "flavin" which had both the biological activity of vitamin B_2 and a close resemblance to the enzyme of Warburg and Christian. Kuhn then determined the chemical composition and structure of this active substance, which he named "lactoflavin," and in 1935 announced its synthesis [807] Lactoflavin or riboflavin, the term adopted by the Council on Pharmacy and Chemistry of the American Medical Association, is composed of iso-alloxazine and ribose.

Riboflavin is phosphorylated in the intestine The ensuing riboflavin-5-phosphate is used to build a number of flavoprotein enzymes [808] Riboflavin-5-phosphate is the prosthetic group in Warburg and Christian's original yellow enzyme [806] and in cytochrome C reductase In all other known flavoprotein enzymes, riboflavin-5-phosphate is united with adenylic acid to form riboflavin-adenine-dinucleotide (FAD), the prosthetic group of a variety of proteins which form the complete enzymes [808] Such enzymes may function in two ways. first, by accepting electrons in the oxidation of TPN or DPN and donating such electrons to oxygen or cytochrome C, and secondly, as direct oxidation enzymes, such as amino acid oxidase or oxidases for specific substrates

The effects of riboflavin deficiency on the tissue content of several specific flavoprotein enzymes have been studied The concentrations of d-amino oxidase are reduced in the liver and kidney of riboflavin-deficient rats;[809] the xanthine oxidase content of the liver is lowered in similarly depleted rats [810] Riboflavin may be demonstrated in tissues by specific histochemical reactions.[811]

Studies of riboflavin-deficient rats have revealed no noteworthy changes in the various non-protein constituents of the blood [812] A moderate creatinuria has been observed, however. A direct relationship has been noted between the protein intake and the riboflavin content of rat liver [813] When dietary protein is reduced, the hepatic content of riboflavin falls, this decrease is independent of the intake of the vitamin Excessive amounts of protein in the diet appear toxic for riboflavin-deficient rats [814] Riboflavin balance is affected by the thiamine content of the diet Chronic thiamine

deficiency leads to an excess of riboflavin in the urine; such a loss is unaccounted for by body tissue breakdown.[815]

The relationship of riboflavin to liver metabolism has been brought out in an interesting series of experiments dealing with the hepatic inactivation of an estrogen, estradiol.[816] When liver slices from animals depleted of riboflavin are incubated with estradiol, they fail to inactivate the hormone, whereas normal liver slices destroy it. In this connection it is of interest that rats receiving large amounts of estrogenic hormone[817] develop *atrophy of the epidermis similar to that which is seen in riboflavin deficiency.*[818] Livers of animals deficient in pyridoxine, pantothenic acid, biotin, or vitamin A retain their ability to inactivate estradiol, while thiamine-deficient animals react in a way similar to those deficient in riboflavin. A relationship of riboflavin to lipid metabolism has been shown; when high-fat diets are fed to riboflavin-deficient rats, such animals survive for a shorter time than those on a high-carbohydrate diet. "Spastic paralysis" of the hind quarters is also observed in such animals.[819]

Further complex interrelations of nutritional deficiency and endocrine metabolism are brought out by experiments dealing with catalase activity of weanling mouse liver.[820] When animals are placed on a riboflavin-deficient diet, the catalase activity fails to show its normal rise to adult levels after two to three weeks. Paired fed controls likewise show this defect. When, however, testosterone is administered, the catalase activity of riboflavin-deficient animals liver rises, while that of the controls does not. Catalase activity of female mouse liver tissue is not affected by riboflavin deficiency.

Riboflavin has been shown to be an essential nutrient for the mouse, rat, cotton rat, hamster, dog, pig, calf and monkey. Prominent changes have been described in the skin, the eyes, and the nervous tissues, as well as in certain isolated organs.

When growing rats are placed on a riboflavin-deficient diet, an initial weight gain is followed in a few weeks by a loss[818] After about six weeks, the fur becomes uneven and ragged, and eventually becomes crusted with a reddish-brown substance. The hair then begins to fall out over the venter. Small, white, dry scales appear along with these changes in the hair. Hair is lost from the eyelids, the lips are erythematous, swollen, and denuded of fur

Microscopically, atrophy of the epidermis and its appendages is found. In the early stages some hyperkeratosis may be seen; no inflammation is present. Most prominent are the changes in the sebaceous glands, whose cells first become swollen, then atrophic. The rudimentary coil glands likewise atrophy. During the early stages, the hair follicles remain normal in appearance. However, the hair which is formed is imperfect. Later the follicular cells become atrophic. Fully developed riboflavin deficiency is

characterized by a skin whose sebaceous glands and hair follicles are atrophic and whose epidermis has decreased in thickness Following therapy with riboflavin the skin changes undergo involution Over the anterior portion of the tongue of the rat the filiform papillae exhibit a defective formation of cornified cells [487]

In the mouse the epidermis microscopically shows either atrophy or hyperkeratosis, there are intra-epithelial accumulations of leucocytes.[821]



FIGURE 77. RIBOFLAVIN DEFICIENCY

Skin, rat A [illegible]

[illegible] there is beginning

Dr. Maurice Sulli-

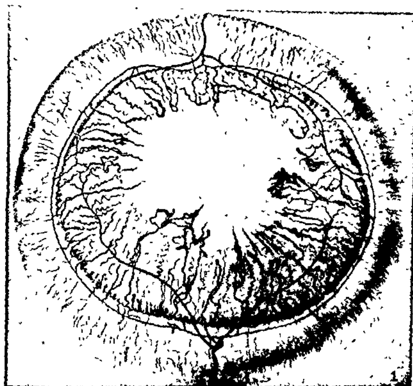


FIGURE 78. RIBOFLAVIN DEFICIENCY.

Cornea There is extensive vascularization of this structure with the ingrowth of many new capillaries. (Courtesy of Dr. S. B. Wolbach and the *Journal of Experimental Medicine*)

The sebaceous glands, in contrast to those of the rat, appear normal. However, the pathogenesis of the skin lesions in this species is not clear and further study is necessary. "Dermatitis" about the mouth has been described in the riboflavin-deficient hamster.[822] When dogs are placed on a riboflavin-deficient regimen, a dry scaling of the skin, accompanied by erythema of the hind legs, chest, and abdomen, has been observed [823] Erythema and scaling of the epidermis have also been reported in swine[824, 825, 826] and in monkeys.[827, 828] Loss of hair and hyperemia of the bucal mucosa is seen in calves.[810] In foxes[829] loss of pigmentation of the fur develops.

Corneal lesions have been described in the rat, mouse, and dog. The changes have been most extensively studied in the first species. In 1939, Bessey and Wolbach[831] carefully described corneal vascularization as a manifestation of riboflavin deficiency. In the rats which they studied, after four weeks on the deficient regimen, capillaries began to grow toward the center of the cornea. The vessels at the limbus seemed to serve as the source

of these sprouting channels. In the ensuing weeks new vessels extended farther and farther, eventually reaching the center of the cornea. The first vessels grew just under the corneal epithelium. The advancing border of the invading vessels was made up of a mass of anastomotic channels with "glomerulus-like loops and arrow-headed-like pointed sprouts" As the deficiency continued, the capillaries penetrated into the tunica propria; however, only in rare instances were vessels found deeper than the junction of the middle and lower third of the tunica. Soon after vascular penetration of the cornea was underway, leukocytes appeared and began to infiltrate the tissue. Changes in the corneal epithelium were not observed during the early stages of the deficiency. Later on, however, although the basal cells remained normal in appearance, the superficial epithelial cells became separated and vacuoles formed between them and the deeper cell layers Descemet's membrane and the endothelial lining of the inner surface of the cornea appeared normal The cornea became progressively cloudy; in the later stages of the deficiency ulceration was found.

Following treatment with riboflavin the turbidity of the cornea rapidly cleared up. Vessels were no longer seen although microscopic study has

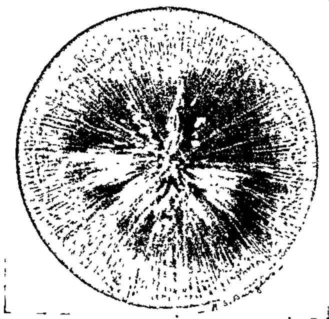


FIGURE 79 RIBOFLAVIN DEFICIENCY.

Lens. Cataract Lens of a riboflavin-deficient swine to show opacities (Courtesy of *Bulletin of the Johns Hopkins Hospital*)



FIGURE 80. RIBOFLAVIN DEFICIENCY.

Congenital malformations. *A* Normal palate of newborn rat in contrast to *B*, cleft palate of animal born to riboflavin-deficient mother. There is a communication between the nasal cavity, nasopharyngeal ducts, and mouth (Courtesy of Dr. Josef Warkany and *The Milbank Memorial Fund Quarterly*)

revealed that collapsed capillaries may be observed in animals for as long as two months following institution of therapy. The above changes, which were thought to be specific for riboflavin deficiency, must now be looked upon as a non-specific response to injury. Corneal vascularization has been shown to be associated with a number of deficient states such as those produced by zinc,[233] protein and various amino acids,[320] pyridoxine,[896] and pantothenic acid.[896]

The Harderian gland of the riboflavin-deficient rat shows fibrosis and leukocytic infiltration.[832]

The lens is a site of damage in several riboflavin-deficient species. Cataracts have been observed in rats,[833, 834] and swine.[824] The first change in the rat consists of a central opacity which spreads peripherally; such cataracts can be arrested by the administration of riboflavin.[833] Although other investigators failed to find such changes in the lens of riboflavin-deficient animals, these discrepancies have been clarified by the demonstration that cataracts do not regularly appear when the diet is completely devoid of riboflavin, but make themselves manifest when small but inadequate amounts of the vitamin are administered.[834] On a riboflavin-deficient diet two of three swine have been observed to develop cataracts after 135 and 145 days.[824] The cataracts in these animals were located in the superficial portion of the cortex of the lens and consisted of "white dot and streak opacities and a few minute vacuoles."

Equivocal changes have been noted in the nervous tissues of mice, dogs, swine, and monkeys. In the mouse myelin degeneration, as evinced by the Marchi stain, has been found in the brachial and sciatic nerves; degeneration in the dorsal columns of the spinal cord has likewise been mentioned.[821] In the dog demyelinization of the dorsal columns of the spinal cord and peripheral nerves has been described.[835] In one of three swine studied by the present writer myelin degeneration of the sciatic and brachial nerves was observed.[834] No histological studies have been carried out in the monkey which is unfortunate, since when M. mullata are placed on a riboflavin-deficient diet, they develop incoordination, a faulty grasp reflex, and loss of strength in the arms and legs,[827] cebus monkeys show no such ill effects[828]

There is some evidence that riboflavin deficiency leads to impairment of red blood cell formation. If rats are first rendered deficient in riboflavin and are then subjected to repeated hemorrhages, a disturbance of red blood



FIGURE 81. RIBOFLAVIN DEFICIENCY.

Congenital malformations. Three [illegible]
A Normal control, B and C [illegible]
or non-separation of ribs. [illegible]
of tibia and fibula The tibia is not present in B. The progressive shortening of the mandible in B and C is also striking (Courtesy of Dr. Josef Warkany and *The Millbank Memorial Fund Quarterly*)

cell and hemoglobin regeneration is found.[836] A mild, microcytic hypochromic anemia is said to develop in dogs,[837] while in swine[824] a moderate normocytic anemia is seen. A reduction in hemoglobin concentration and red blood cell count has been observed in monkeys.[827] In all of these hematological studies the data are too inadequate to permit any general conclusions.

The fat content of the liver (per dry weight) is increased in riboflavin-deficient dogs from a normal of about 15 per cent to 40 or 50 per cent.[823] Similarly, two of three deficient swine have exhibited on microscopic examination rather large quantities of fat in the liver, and in all, the convoluted tubules of the kidney contained globules which could be stained with Scharlach R.[824]

For the past years Warkany and his associates[838, 839] have been studying the effects of maternal nutritional deficiencies on their offspring. In rats, shortening or absence of the tibia, mandible, fibula, radius, ulna, femur, ribs, fingers, and toes have been observed. Fusion of the ribs and cleft palate may also accompany the above changes, all of which have been shown to be prevented by the inclusion of riboflavin in the maternal diet before or on the 13th day of gestation. After this critical period abnormalities will appear in the newborn whether or not riboflavin is administered. It should be pointed out that, although the diet first used by Warkany was not a purified one, consisting as it did of cornmeal, wheat gluten, sodium chloride, and calcium carbonate, supplemented with crystalline vitamins, conclusive results showing that riboflavin is the protective factor have been obtained on synthetic rations composed of sucrose, casein, fat, salts, and crystalline vitamins. Skeletal abnormalities have been described by others.[840, 841] The ratio of calcium to phosphorus in the diet is important in their production.[841]

The riboflavin-deficient rat exhibits an abnormal reaction to decreased oxygen concentration.[842] No new formation of glycogen occurs in the liver in comparison with a ten-fold increase in controls. Hence, adrenocortical failure may be a part of the riboflavin deficiency syndrome.

Mention should be made of the relation of riboflavin to spontaneously occurring and to chemically induced tumors. When rats are fed amino-azo dyes, those having high dietary levels of riboflavin are less likely to develop liver tumors than those having lower concentrations of the vitamin in their rations.[843] The riboflavin protecting effect on the liver may be related to the ability of the hepatic cells to destroy the carcinogen.[844] When a tumor induced by an azo-dye is transplanted, its growth is enhanced by plenty of dietary riboflavin.[845] These are but a few of the experiments on tumor growth in which the effect of riboflavin has been studied.

Riboflavin deficiency in *man* has recently been studied in fifteen adult male subjects at the Elgin State Hospital[846] A diet furnishing 2,200 calories and containing .55 mg of riboflavin was administered for periods from nine to seventeen months. During this time certain clinical signs developed in all but three subjects: angular stomatitis, seborrheic dermatitis and scrotal skin lesions. The previous studies of others[847] are also reviewed by the Elgin group.

The skin was the site of most prominent change. The most common area was the scrotum This began as a patchy erythema associated with scaling which was followed by desquamation of the superficial epithelial layers of the anterior surface. The course was one of exacerbations and remissions. With therapy the healing response was dramatic.

An itching seborrhea occurred in several subjects This was found over the scalp and chest. In addition cheilosis, involving the vermilion border of the lips with vertical fissures, crusts, and desquamation was observed in one subject.



FIGURE 82. RIBOFLAVIN DEFICIENCY.

Angular stomatitis which developed on riboflavin-deficient regimen in thirty year old male. (Courtesy of Dr M. K. Horwitt)



FIGURE 83. RIBOFLAVIN DEFICIENCY.

Scrotum. A and B Scaly dermatitis which was observed in subjects on riboflavin-deficient regimen (Courtesy of Dr. M. K. Horwitt.)

Comment should be made concerning certain negative findings. No corneal vascularization, glossitis, capillary abnormalities or neurological abnormalities were noted.

NIACIN

Although nicotinic acid had been prepared synthetically in 1867 and subsequently was demonstrated to occur in many foodstuffs, its importance in nutrition did not become apparent until 1935. In that year nicotinic acid amide (nicotinamide) was shown to be an important constituent of two already well-known co-enzymes. Warburg[848] demonstrated that the "hydrogen-carrying enzyme of red blood cells," or Co-enzyme II, consisted of adenine, a pentose, phosphoric acid, and nicotinic acid amide. Shortly thereafter, Euler and his co-workers[849] showed that cozymase or Co-enzyme I likewise contained the amide of nicotinic acid. When Elvehjem and his group[850] demonstrated in 1937 that nicotinic acid or its amide was effective in curing blacktongue in dogs, these materials came into widespread use in the treatment of pellagra in humans. Nicotinic acid is pyridine 3-carboxylic acid

Ingested nicotinic acid is transformed *in vivo* into the amide, this is utilized to form the coenzymes mentioned above These materials are heat-stable, dialyzable, organic substances which function as hydrogen carriers in cellular respiration.[808] Their chemical natures are similar Coenzyme I or diphosphopyridine nucleotide (DPN) contains one mol less of phosphoric acid than does Coenzyme II or triphosphopyridine nucleotide (TPN). Coenzyme III, which was discovered later, has no adenine molecule and only one mol each of ribose and phosphoric acid[808] DPN and TPN function as acceptors of hydrogen in association with a large number of specific enzymes and their substrates[808] Some examples are ethyl alcohol dehydrogenase) + DPN→acetaldehyde, vitamin A (alcohol dehydrogenase) + DPN→retinene$_1$, isocitric acid (isocitric dehydrogenase) + TPN→alpha ketoglutarate.

DPN may be demonstrated in the tissues. For instance, liver, muscle and kidney cortex of normal dogs contain appreciable amounts.[851] Only in the liver are concentrations of DPN reduced appreciably when nicotinic acid deficiency is induced

Knowledge of the indispensability of nicotinic acid was in a confused state for several years after this material was shown to benefit the blacktongue syndrome in dogs (page 329) and the pellagra syndrome in man (page 316) Although Frost and Elvehjem[852] had found that nicotinic acid supplements improved growth of rats fed a diet which was inadequate in certain respects, it was soon shown that, when this species was placed on an optimal diet, nicotinic acid was not necessary.[853] A similar situation was found in the dog[854] and in swine.[855] Hence, difficulties arose in trying

to explain the beneficial effects of nicotinic acid under certain circumstances. It is not necessary to go into the entire story of how this problem was solved. The quantity and, in particular, the quality of the protein proved to be the answer. In conclusion, tryptophan was demonstrated to be the biological precursor of nicotinic acid (page 89).

Certain dietaries would appear to increase the requirement for nicotinic acid. Corn is, of course, notorious in this respect because of its low tryptophan content.[1214 1215] Furthermore, it has been suggested that corn contains some material, a "pellagragenic factor," which increases the need for nicotinic acid or which may act as an antimetabolite of niacin.[856]

The tryptophan-niacin relationship is analagous to that of methionine and choline Hence, in studying nicotinic acid deficiency, the amounts of tryptophan in the diet must be kept at a minimum so as to have as little conversion to niacin as possible. Deficient states have been described in experimental animals [1214 1215] and in man when diets low in tryptophan content and deficient in nicotinic acid were fed. When calves are placed on such a regimen as early as twenty-four hours after birth, they develop diarrhea within forty-eight hours.[857] They soon become dehydrated and after several more days are unable to stand. Death occurs suddenly. When nicotinic acid is administered, prompt improvement ensues. Similar findings have been reported in young swine.[858]

Acetyl-pyridine, which is assumed to be an antagonist to nicotinamide, has been administered to mice and rats in order to determine whether any morphological lesions might be elicited.[859] Evidences of damage to peripheral ganglion cells, and to the neurons in various areas of the brain and spinal cord were found. Such alterations are of interest in view of the lesions which have been described in pellagra in the human (page 326).

The relation of corn to the pellagra syndrome and the curative effects of nicotinic acid on certain manifestations of this disease are well-established (page 316) Hence, experimental studies of humans which have been placed on corn diets low in niacin content are valuable. Goldsmith and her co-workers [860 861 862] have contributed a series of reports on the niacin requirement of man, utilizing diets of maize which were low in tryptophan and niacin content, diets of wheat and corn, and diets containing lime-treated and untreated corn. These important studies connot be recounted in detail [illegible]
were maintained on [illegible]
save that it containe[illegible]
developed clinical signs of pellagra after fifty days. Such manifestations were dermatitis, cheilosis, angular stomatitis, lesions in the nasolabial folds, diarrhea, glossitis, amenorrhea, mental depression and apathy. The distribution and gross appearance of the dermatitis was characteristic of pellagra.

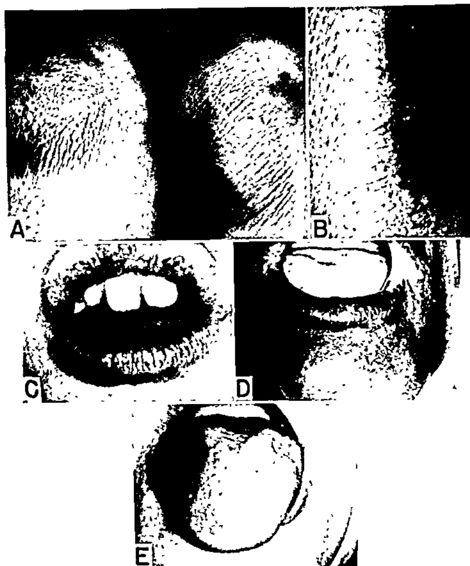


FIGURE 84 TRYPTOPHAN-NIACIN DEFICIENCY

A and *B* Scaling dermatitis of exposed surfaces which developed in subjects on diet low in tryptophan and niacin *C* and *D* Lesions and angles of mouth which were observed in subjects on a tryptophan-low niacin diet. *E* Smooth tongue which developed on same regimen (Courtesy of Dr Grace Goldsmith)

The face, hands, vulva, perineum and perianal regions were involved. Glossitis was prominent. Burning sensations in the esophagus were noted Diarrhea developed. In all three of the subjects, the excretion of n-methylnicotinamide was decreased.

PANTOTHENIC ACID

In 1933, Williams and his associates[863] announced the isolation of a new growth factor for yeast Since this material was an acidic substance, which could be demonstrated in a wide variety of living cells, it was named "pantothenic" (derived from the Greek "from everywhere") acid. During the next few years work went forward on the occurrence, chemistry, and biological activity of the new compound, so that by 1940 Williams' laboratory was able to announce the synthesis and the chemical structure of biologically active pantothenic acid.[864, 865]

From the very beginning of his experiments Williams had expressed the belief that pantothenic acid was a water-soluble vitamin; in fact, he had suggested in 1933 that the material might be related to vitamin G (riboflavin).[863] Not until 1939, however, was pantothenic acid shown to be an antidermatitis factor for the chick. SubbaRow and Hitchins[866] then demonstrated that this compound was a growth factor for the rat.

The story of the development of our knowledge of the metabolic role of pantothenic acid is one of the most fascinating aspects of nutritional research during the past decade[867] It culminated in the demonstration, by "a number of fortunate accidents," that pantothenic acid is an important part of coenzyme A (CoA), a key substance in intermediary metabolism The transformation of pantothenic acid to CoA has been worked out.[868] CoA is important for the integrity of adrenal function, acetycholine synthesis, acetylation of various materials, formation of certain lipids such as cholesterol, and, of course, the Krebs cycle.[861]

As might be expected, the CoA concentration is decreased in the tissues of pantothenic acid-deficient animals (rats)[869] The plasma cholesterol content is reduced in this deficiency as well, though fat must be excluded from the diet if this effect is to be demonstrated[870] The administration of growth hormone to adult rats which have been placed on pantothenic acid-deficient diets precipitates the effects of deficiency.[871]

The indispensability of pantothenic acid has been demonstrated for the rat,[872] mouse,[873, 874] guinea pig,[877] hamster,[822] cotton rat,[876] dog,[892] pig,[879] calf,[886] fox[829] and monkey.[878] Microscopic studies of tissues have not been performed on all of these species.

Specific lesions in the skin and hair have been described in the rat, their pathogenesis has been carefully studied by Sullivan and Nicholls.[872] First, a circumocular loss of hair (spectacle alopecia) is noted. The hair is also lost in the preauricular region and along the sides of the snout. This alopecia is sometimes accompanied by scaling. Graying of the hair



FIGURE 85. PANTOTHENIC ACID DEFICIENCY.

Hair. Head of rat which had been on a pantothenic acid deficient diet for about five weeks Note symmetric graying (achromotrichia) of hair about eyes, ears, and nose This usually spreads to involve the entire head, later alopecia occurs. (Courtesy of Dr. Maurice Sullivan and the *Archives of Dermatology and Syphilology*)

has been observed in piebald rats, being prominent in the circumocular regions, sides of the nose and over the shoulders. The fur becomes dull and coarse The graying (achromotrichia) is followed by a generalized scaling and erythematous dermatitis. Occasionally, foci of eczematous dermatitis are observed. Following these epidermal changes the hair begins to fall out.

Microscopically, moderate hyperkeratosis and acanthosis are seen, together with an occasional focus of intraepidermal vesciculation and crusting, especially in areas where small eczematous foci had been noted grossly. As the rats become more depleted in the vitamin, the epidermis approaches

its usual thickness or even becomes atrophic. A consistent change is found in the hair follicles, whose lumens become dilated from orifice to bulb. The hair is lost at this time. Changes in the sebaceous glands are usually insignificant until the terminal stages of the deficiency when these structures undergo atrophy Little cellular infiltration is found in the corium at any time

The achromotrichia which has been observed in pantothenic acid-deficient rats has been confirmed and further clarified by observations which have shown that pantothenic acid is not the only chromotrichia factor.[875] Copper deficiency (page 62) and lysine deprivation[354] also lead to graying of the hair, evidence for the existence of a fourth factor, para-aminobenzoic acid, has also been presented (page 269). It is of interest that the achromotrichia in pantothenic acid-deficient rats may be reversed by adrenalectomy.[882]

In the skin of mice, hyperkeratosis, followed by atrophy of the epidermis, has been described[873] There is no inflammatory reaction. No mention has been made of the condition of the hair follicles or sebaceous glands. Alopecia has been reported by others.[874] A "red incrustation" has been described about the mouth of hamsters,[823] while the cotton rat exhibits an unspecified dermatitis[876] Hair color of the guinea pig is dulled,[877] graying



FIGURE 86 PANTOTHENIC ACID DEFICIENCY.

Skin Section of skin from head of rat which had developed alopecia. Note dilatation of the hair follicles which is characteristic of this deficiency. No changes are found in the sebaceous glands, corium, or epithelium (Courtesy of Dr Maurice Sullivan and *Archives of Dermatology and Syphilology*)



FIGURE 87. PANTOTHENIC ACID DEFICIENCY

Colon, pig *A* Normal mucosa of colon. *B* Tissue from deficient animal which had acute diarrhea. Note superficial ulceration, leukocytes in lumen of glands and interstitial infiltration *C* Tissue from chronically deficient animal. Note loss of epithelial glands, extreme interstitial infiltration and chronic ulceration. All H and E (x 80).

and alopecia are seen in the monkey.[878] Alopecia occurs in swine, on microscopic examination atrophy of the epidermis and loss of hair follicles are found.[879]

Lesions of the mouth have been described in pantothenic acid-deficient rats. Such changes consist of ulcers, hyperkeratosis of the oral mucous membranes, and marked gingival and peridontal necrosis.[880]



FIGURE 88. PANTOTHENIC ACID DEFICIENCY

Colon, pig Lesions in the solitary follicles of the colon A Solitary lymphoid nodule from pantothenic acid-deficient pig showing the normal prolongation of glandular elements into this structure There is beginning leukocytic infiltration of the glands The lymphoid tissue is somewhat hyperplastic *B* More advanced lesion which has become two abscesses in the middle of the follicle. Such abscesses grow and suppurate, producing ulcers. Both H. and E. (x 25) (Courtesy of the *Bulletin of the Johns Hopkins Hospital.*)

Diarrhea is an early and constant sign in pantothenic acid-deficient swine.[879, 883, 884] The stools frequently contain mucous and sometimes blood. Rectosigmoidscopic examination reveals a diffusely hyperemic mucosa which is slightly edematous. Bleeding usually occurs as a result of instrumentation. Ulceration has not been detected by this method of examination, however.

At autopsy, extensive changes are found in the intestine, particularly the colon. Grossly, the earliest change is a diffuse hyperemia which may appear after four weeks of the deficient regimen. The lymphoid follicles are enlarged and on section exhibit purulent centers. Perforation of such abscesses lead to small ulcers which then become confluent. The mesenteric lymph nodes are enlarged. Microscopically, one finds a change from the normal glandular mucosa, which is made up of large vacuolated cells, to a lining composed of glands formed by atrophic cells. Leukocytes are found in the lumina of these glands, as well as in the interstitial tissues about them. Although, in the early stages, this alteration is a focal one, as time goes on it becomes more and more diffuse. Cells accumulate in the glands and these structures become progressively dilated. As a result, the atrophic epithelial cells appear even more flattened. In the lymphoid follicles, which also contain glandular prolongations in their centers, the same lesions are found Here, following necrosis of the epithelium abscesses develop; these finally rupture, leaving large ulcers After treatment with calcium pantothenate, such ulcers heal. In these cases the intestinal wall at autopsy is found to be thickened, due to an increased proliferation of connective tissue, which apparently results from the previous tissue destruction and healing Changes have not been described in the intestine of other species.

During life pantothenic acid-deficient swine evince a disturbance in gait. This is first displayed by a sudden elevation of one of the limbs from the ground as though it were painful. The gait next exhibits a broadening base and a jerky "goose step" appears. As the deficiency progresses, the gait becomes more and more impaired, so that finally the animal is unable to walk at all and lies prostrate.

Microscopic examination[885] reveals that the earliest change is chromatolysis of the dorsal root ganglion cells. These alterations have been observed in animals in which ataxia had not been detected during life. The ganglion cells exhibit the classical signs of disintegration and lysis of the Nissl substance, cells of all sizes appear to be equally involved. When the spinal or peripheral nerves of such animals are examined by appropriate techniques, no changes are found. However, later, that is from the eighth week of the deficiency on, loss of myelin and axis cylinder degeneration are found in the brachial and sciatic nerves. As time goes on, changes may likewise



FIGURE 89 PANTOTHENIC ACID DEFICIENCY

Sensory ganglia, pig *A, B, C* Dorsal root ganglion cells to show chromatolytic phenomena, i e, loss of Nissl substance or its condensation about periphery of cell All sizes appear to be involved In *C*, cells have died and collections of mononuclear are found in their place At the stage of changes seen in *A* and *B* the peripheral nerves are normal *A* (x 265), *B* (x 335), *C* (x 300)

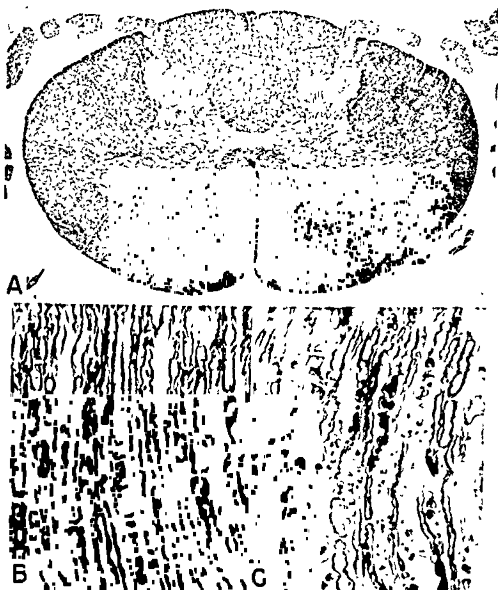


FIGURE 90. PANTOTHENIC ACID DEFICIENCY

Spinal cord, pig *A* Lumbar segment from animal dying after ninety-nine days on deficient regimen. Note loss of myelin in dorsal columns *B* and *C* sections of ventral and dorsal roots, respectively, to show loss of myelin in latter and no change in former.

be observed in the dorsal root fibers, and in a single animal, degeneration of some of the fibers in the dorsal columns has been noted. Chromatolytic cells have been found in the anterior and intermediate gray matter of a small number of animals. Using the osmic acid technique, which is notoriously unreliable, myelin degeneration has been reported to be present in the dorsal columns and pyramidal tracts of the spinal cord and in the peripheral nerves of pantothenic acid-deficient mice.[873] Weakness of the extremities has been noted in deficient calves[881] Ataxia has been reported in monkeys[878]

A manifestation of pantothenic acid deficiency in rats was first described as "blood-caked whiskers" The nose and hairs of the snout become covered with a reddish pigment The Harderian glands have been shown to be the source of this material, which is said to be corproporhyrin The pigment appears to be excreted through the nasolacrimal duct in pantothenic acid-deficient animals, for when the Harderian glands are excised and the animals are then placed on a pantothenic acid-deficient regimen, the chromodacryorrhea fails to appear[886] Dehydration may also be a factor leading to pigment incrustation of the nose and whiskers of rats.[887]

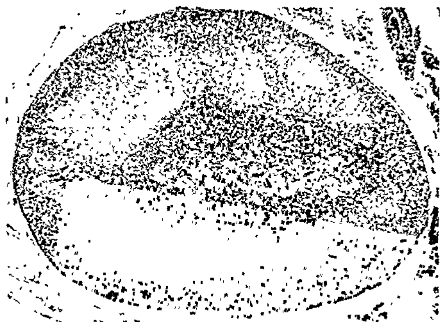


FIGURE 91 PANTOTHENIC ACID DEFICIENCY

Adrenal, rat Entire section of adrenal gland from rat on pantothenic acid-deficient diet Note intact medulla and inner reticular zone Outer reticular zone, entire fascicular and inner glomerulosa are necrotic

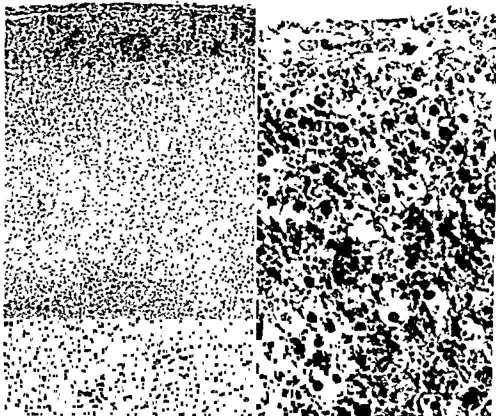


FIGURE 92 PANTOTHENIC ACID DEFICIENCY

Adrenal, rat A. Low power (x 35) and B higher magnification (x 200) of cortex of adrenal gland Virtually all, save the outer glomerulosa, is necrotic.

One of the most interesting manifestations of pantothenic acid deficiency is the appearance of so-called "hemorrhagic necrosis" of the adrenal glands in rats.[882, 889] The pathogenesis of this lesion has been carefully studied.[890] The first change, which occurs in the first few weeks of the deficiency, is a loss of lipid material (sudanophilic) from the cortex. Histochemical procedures would appear to demonstrate loss of ketosteroid compounds These changes begin in the inner cortex and extend outwards. Associated with such alterations mitotic figures are found in the cells of the outer zona fasiculata. Next necrotic cells appear in the reticular region. From the second week on, increasing necrosis and hemorrhage are seen. In severely affected glands the zona glomerulosa may be involved The medullary portion of the gland does not appear to be affected. Alkaline phosphatase activity of the cortical cells is decreased.[890]

Extremely interesting studies of adrenal behavior in pantothenic acid-deficient animals have been carried out following the administration of ACTH or cortisone[891] When the pituitary hormone is given, the adrenal lesions tend to be more severe On the other hand, cortisone tends to prevent the development of the lesions Because pantothenic acid-deficient rats exhibit a disturbance in carbohydrate metabolism, i e, hypoglycemia, the view has been advanced that a deficiency of this vitamin leads to adrenal functional insufficiency. The possibility that CoA is a necessary factor for the biosynthesis of steroids is thus a real one

Adrenal enlargement, with hyperemia and hemorrhage, has been described in the guinea pig[877] The only possible evidence of any alteration in the adrenal glands besides the rat and guinea pig is a dramatic syndrome which has been described in dogs[892] After a variable period, depending on the vitamin intake, there appear. sudden prostration or coma, tachypnea and tachycardia, convulsive movements of the extremities, and vomiting Death ensues unless treatment is instituted Chemical studies of the blood have revealed an irregular lowering of glucose and chloride concentrations, together with an increase in non-protein nitrogen values Gross findings at autopsy have been equivocal, except for light colored livers whose fat contents on chemical analysis range from 34 7 to 55 1 per cent in contrast to the normal ranges of 13-17 per cent Microscopic studies have not been reported. In another experiment on dogs, fatty livers and spasticity of the hind quarters have been noted but no examination of the tissues has been reported[893]

As might be expected, reproduction may be severely affected, this has been studied in rats[894] and in swine[895] In the former species, when pantothenic acid deficiency was instituted sixteen to twenty-three days before mating, there resulted failure of implantation, resorption, or defective litters. This effect was not due to inanition Newborn animals are also affected.[901]

A few other miscellaneous effects of pantothenic acid deficiency have been noted Some appear to be non-specific, i.e, corneal vascularization[896] and disturbance in endochondral bone formation[897] On the other hand, granulocytosis without lymphopenia has been noted in mice[898] In dogs the elevated blood pressure produced by renal ischemia has been returned to normal as a result of pantothenic acid deficiency.[899] The synthesis of antibodies appears to be related to pantothenic acid since there is a decrease of this reaction in deficient animals. However, antibody formation does not appear to answer all the questions raised by changed resistance to infection, for the natural resistance of the rat to a strain of *Cornybacterium kutscheri* is abolished by pantothenic acid deficiency even though antibody production does not appear to be particularly affected[900]

Three human subjects have been fed a pantothenic acid-deficient diet

to which the antagonist, omega-methyl-pantothenic acid was added.[902, 903] These experiments were acute and rather severe. They showed that certain effects could be produced and suggest that pantothenic acid is an essential nutrient for man. The subjects developed a neuromotor disorder, cardiovascular instability, gastrointestinal complaints, repeated infections, and physical and mental depression. All of these disturbances were relieved when the missing nutrient was added to the diet. The neurological changes consisted of numbness and tingling of the hands and feet. One subject complained of burning of the feet. All had hyperactive deep tendon reflexes and weakness of the extensor muscles. No sensory disturbances were noted as far as pain, temperature or vibration were concerned. Certain biochemical changes which developed are of interest. These consisted of alterations in acetylation, increase in insulin sensitivity, lack of eosinophile response to ACTH, hypocholesterolemia, decrease in 17-ketosteroid excretion and depression of gastric secretion with respect to hydrochloric acid production Gastric motility was not impaired.

VITAMIN B_6 GROUP

In 1926, Goldberger and Lillie[904] described a "pellagra-like" condition in rats which had been placed on a diet composed almost entirely of corn-meal which had been extracted with alcohol Striking lesions appeared in the form of a bilateral, symmetric, scaly dermatitis which involved the extremities, ears, and face, the trunk was only occasionally affected. Since the skin changes could be prevented by autoclaved yeast, it was assumed that they were caused by a deficiency of vitamin B_2 (as the heat-stable portion of the B group was then called). To Gyorgy and his associates[905] goes the credit for showing that riboflavin (vitamin B_2 or lactoflavin) did not cure this "pellagra-like dermatitis," and that another factor—vitamin B_6 was necessary Gyorgy suggested that the new dietary essential should be called the "rat acrodynia factor" since the lesions of the extremities resembled those observed in human acrodynia, rather than the changes of pellagra

In 1938, a crystalline material was isolated, the hydrochloride of a nitrogenous base, this had the properties of György's vitamin B_6 and was soon shown to be 2 methyl-2 hydroxy-4, 5-di (hydroxymethyl) pyridine The synthesis of vitamin B_6 was then announced Gyorgy suggested that, in accordance with the chemical nature of vitamin B_6, which is a pyridine derivative containing several oxy (methoxy) groups, the term "pyridoxine" appeared to be appropriate

At the present time not one but three members of the vitamin B_6 group are recognized. In addition to pyridoxine, pyridoxamine and pyridoxal exhibit comparable biological activity When fed to man these three forms appear to be metabolized in similar fashion, each forming pyridoxal, which is utilized as the phosphate in metabolic reactions[906] Each is excreted as 4-pyridoxic acid Pyridoxal-phosphate is a codecarboxylase for at least six specific amino acids.[908] Another general reaction, transamination, is dependent on this vitamin. For instance, a decrease in alanine-glutamic transaminase activity is found in the liver of vitamin B_6-deficient rats[908] So, too, blood urea levels are elevated in such animals

Pyridoxine is concerned with the metabolism of tryptophan. When the urine of pyridoxine-deficient rats is treated with ferric ammonium sulfate, a green pigment appears[909] This substance was identified some years ago as xanthurenic acid, an intermediary in tryptophan metabolism.[910] Further studies in rats showed that pyridoxine was necessary for the metabolism of xanthurenic acid[911] This finding has been confirmed in swine, in which the appearance of xanthurenic acid in the urine can be correlated with

the onset of a characteristic anemia, which develops in this species[913] Tryptophan is metabolized to kynurenine, which is further transformed to 3-hydroxyanthranilic acid and thence to nicotinic acid.[328] (see page 89).

These important roles of pyridoxine in protein metabolism go far to explain some early observations on the relation of the protein content of the diet to the development of deficiency signs in the rat. When such animals are placed on a high protein (45 per cent, 30 per cent) diet, they show the characteristic skin lesions sooner and die in a shorter time than animals whose pyridoxine-deficient diet contains a lower amount (15 per cent) of protein.[914] The high protein diets would not appear to be harmful because of their tryptophan content.[915]

The vitamin B_6 group is intimately concerned with fat metabolism. Skin lesions are said to appear more readily in pyridoxine-deficient rats if their diets are also lacking in essential fatty acids. Moreover, the total fatty acid content of the carcass of the vitamin B_6-deficient rat is lower than that of control animals, even though the diet has contained abundant lipids.[916]

Studies of pyridoxine deficiency have been reported in the following species: rats, mice, hamsters, cotton rats, cats, dogs, foxes, pigs, cattle, and monkeys The most prominent changes have been found in the skin, erythropoietic tissues, and nervous tissues. Miscellaneous lesions have been noted in other organs

In the rat,[917, 918] the skin is the most prominent site of injury. Grossly, the initial change is an erythema of the dorsa of the paws, most commonly the hind ones. This reddening soon spreads to the plantae and is followed by hyperkeratosis and scaling. Next the digits become swollen. Coincident with the development of these changes in the extremities, the same process appears in the ears, nose, chin, submental region, and upper thorax. The coat looks ill-kept, alopecia develops late in the course of the deficiency.

Microscopically, hyperkeratosis and acanthosis, together with erythema and edema of the corium are prominent. Leukocytes infiltrate the corium. The sebaceous glands and hair follicles remain unaffected until late in the course of the disease. It is probable that these accessory structures are damaged as a result of secondary infection coincident with epithelial ulceration,[917] though the changes in the sebaceous glands and hair follicles have been considered by some to be a late primary effect.[487, 916] It has recently been suggested[919] that all of the epithelial changes are secondary to the vascular alterations.

The skin lesions, particularly the initial changes in the extremities, have aroused much interest, especially in relation to the distribution of the dermatitis in human pellagra. No effects on the dermal lesions have been produced either by excessive sunlight or by denervation.[487] It has been

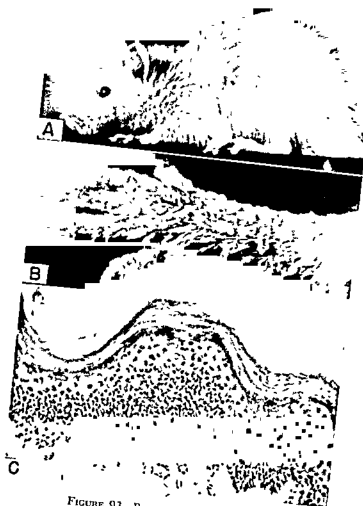


FIGURE 93. PYRIDOXINE DEFICIENCY

Skin, rat. *A*, External appearance of rat after being on a pyridoxine-deficient regimen for about six weeks. Note normal appearance of fur in contrast to riboflavin-deficient animal, Figure 77, page 211. At this time the only change, aside from some failure to grow, is found in the extremities where scaling, *B*, is noted. Microscopically, *C*, note hyperkeratosis and acanthosis with epithelial proliferation just above the basal cell layer. (Courtesy of Dr. Maurice Sullivan and the *Journal of Investigative Dermatology*.)

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should be extended, however, to the dog and swine, since these species exhibit a much more marked disturbance in hematopoiesis Both puppies and adult dogs tend to develop an anemia, which is improved by the administration of pyridoxine[927, 928, 929 931] However, normal red cell and



FIGURE 95. PYRIDOXINE DEFICIENCY

Spleen, pig A Section of spleen from a pig sacrificed after being on a pyridoxine-deficient diet for 139 days Significant anemia developed in forty-seven days and maximal anemia was present eight days before death RBC, 3,650,000, M.C.V , 43 cu μ, MCHC, 24 per cent (see text for normal values) The bone marrow was hyperplastic and there was hemosiderosis of this tissue as well as the liver, characteristic lesions were found in the sensory neurons This section illustrates the extensive deposition of hemosiderin pigment in the pulp, capsule and trabeculae. Note that the Malpighian bodies are spared and compare with *B*, where the Malpighian bodies stand out as darker groups of cells from the lighter staining surrounding pulp No pigment is seen in this pulp, there is, however, some pigment in the capsule This animal had been severely anemic at one time. Following treatment with pyridoxine there was a sharp reticulocyte response and the blood returned to normal At autopsy no hemosiderin was found in the bone marrow, liver, or spleen save for the remnants of the pigment which is seen in the capsule. Both Prussian blue, fucsin stain (x 35).

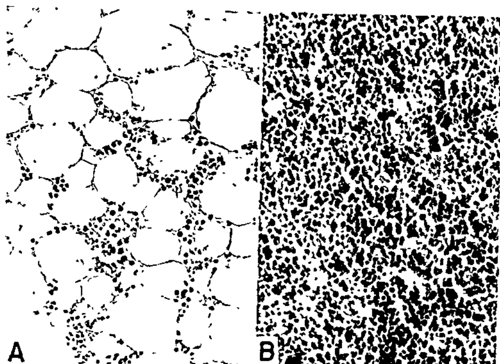


FIGURE 94. PYRIDOXINE DEFICIENCY.

Bone marrow, pig. *A* Section of normal femur to show small islands of erythro- and myelopoetic elements *B* Section from pyridoxine-deficient swine to show extreme hyperplasia. No fat remains. This animal had been on the deficient diet for 102 days Wright stain (x 235).

reported that the skin changes appear earlier in rats exposed to a cold environment,[920] this is likely due to a heightened general metabolism with increased need for the vitamin, rather than to any localized change in the extremities.

"Acrodynia-like" lesions have been noted in pyridoxine-deficient mice [921, 922] On a pyridoxine-deficient diet the Syrian hamster is said to develop dermatitis about the mouth.[923] Specific skin changes have not been a prominent feature of pyridoxine deficiency in the other species studied.

In some rats deficient in pyridoxine, an anemia has been found. Disturbances in red blood cell formation can be more definitely demonstrated if such animals are bled in addition, for then real impairment of red blood cell regeneration develops.[924, 925] Because of this relationship of pyridoxine to hemoglobin formation in the rat, the catalase content of tissues from deficient animals has been studied. It will be recalled that this enzyme is an iron-porphyrin compound like heme. No decrease in the catalase content of the liver, kidney, or heart muscle has been found [926] Such studies

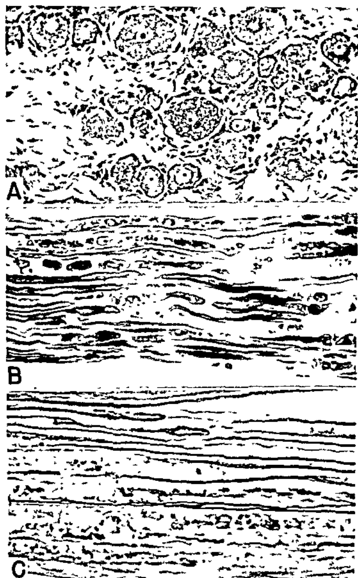


FIGURE 96 PYRIDOXINE DEFICIENCY

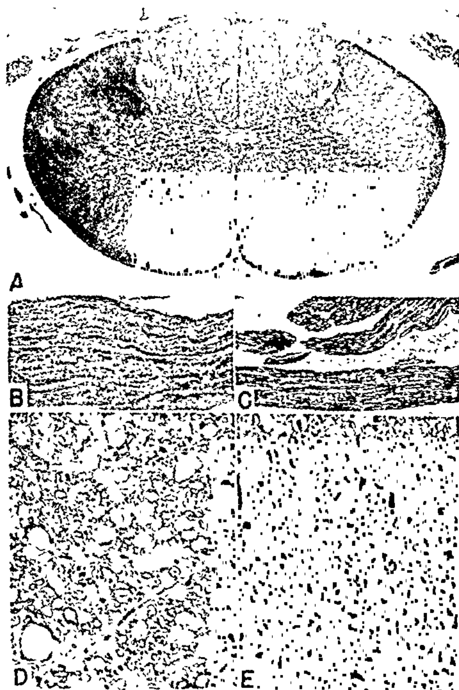
Sensory neuron and nerve, pig A Dorsal root ganglion cells which show no chromatolysis. There is atrophy B Myelin stain and C silver stain to show degeneration of myelin and loss of axis cylinders

hemoglobin levels are usually not obtained unless liver is given as well The anemia is characterized as microcytic and hypochromic; elevated plasma iron levels are observed as the blood changes progress. The anemia and its response to vitamin B_6 should be restudied now that folic acid and *vitamin B_{12}* are available.

In swine, an anemia is observed after animals have been on a pyridoxine-deficient regimen from four to six weeks.[912] Once significant anemia appears, it usually progresses in a few weeks to one of severe degree; for instance, from a normal count of 8,000,000 red cells to one of 3,200,000 per cubic milliliter of blood. The anemia is primarily microcytic in type. Values of 40 cubic microns or less for the mean corpuscular volume have been observed (normal for swine is about 58 cubic microns). The normal mean corpuscular hemoglobin concentration of 33 per cent is little, if any, reduced. Anisocytosis is marked, and an irregular reticulocytosis is usually present. No increase in the icterus index or any decreased resistance to hemolysis in hypotonic saline solution may be demonstrated. Following treatment with pyridoxine, an immediate increase of circulating red blood cells occurs; the cells then return to their normal size. As in the dog, pyridoxine does not completely relieve the anemia in swine, it must be assumed that other unknown factors are necessary. As the anemia of pyridoxine deficiency develops, a rise in serum iron content to values as high as 300 micrograms per 100 cc. is observed. Upon treatment the serum iron concentration falls to the normal value of 100 micrograms or less.

During the course of the anemia in swine an extensive deposition of iron pigment, as demonstrated by the Prussian blue reaction, is found in the liver, spleen, and bone marrow. This pigment, presumably hemosiderin, occurs both intra- and extracellularly in the splenic pulp, as well as in the capsule and trabeculae. Virtually no pigment is found in the Malpighian bodies. The Kupffer cells of the liver contain pigment, and in those animals dying with severe anemia the periportal cells of the liver lobule are also filled with iron-staining material. Macrophages in the bone marrow of anemic animals are loaded with pigment. None has been observed in the renal tubular epithelium. The bone marrow of anemic animals is hyperplastic and contains numerous "blast" cells, as well as nucleated red cells. Treatment with pyridoxine diminishes the amount of iron pigment in the spleen, liver, and bone marrow; the duration of treatment can be correlated with the amount of pigment remaining in the splenic pulp. Some iron-staining material remains in the capsule and trabeculae even after prolonged therapy

The ch[illegible]
with thosc
ciency.[932] [illegible]


A
B
C
D
E

since elevated serum bilirubin and increased excretion of urobilinogen in the urine and feces and of porphyrin in the urine, all of which may be noted in phenylhydrazine hemolytic anemia, are not observed in pyridoxine-deficient animals. It is concluded that the hyperferremia and hemosiderosis are due to the continual absorption of iron at a time when its utilization for hemoglobin is at a minimum, and when the iron content of the tissues is abundant. Elevated serum iron levels and hemosiderosis of the tissues do not occur in rats deficient in both iron and pyridoxine.[933]

Epileptiform fits, which lasted several minutes, were first described in pyridoxine-deficient rats by Chick as follows:[934] "(1) A violent stage in which the rat would suddenly rush about wildly with protruding eyes, jumping to the floor of the room if not restrained and leaping up into the air, sometimes uttering cries; this stage usually lasted less than thirty seconds In a few instances the eyes became suffused with blood, which drained away through the nasolacrimal ducts. Occasionally the rat urinated during the fit, and on one occasion vomiting of stomach contents was observed. (2) A helpless condition in which there were muscular twitchings and tonic spasms while the rat lay helpless. Sometimes the digits of one of the forepaws clasped with those of the hind paw of the same side. (3) A comatose condition when the rat sometimes became unconscious, with a slowed and weakened heartbeat and absence of corneal reflex. (4) Gradual recovery, control being regained first of the forepart of the body and later of the hind limbs" More specific studies of these vitamin B_6 induced fits in rats have revealed that the animals show an increased brain excitability as measured by a decrease in electroshock threshold. Administration of pyridoxine and/or glutamic acid tend to raise the threshold; on the other hand, tryptophan, as might be expected, reduces it.[935] Such observations may be explained by the important role of glutamic acid in the metabolism of the brain. Moreover, cerebral tissue contains an active glutamic decarboxylase which needs pyridoxal phosphate for its activity.[939]

Epileptiform seizures have been observed in deficient puppies, but are apparently uncommon in adult dogs. Pyridoxine-deficient swine show two manifestations of neurological damage during life: convulsions and ataxia.[936] Convulsions may appear as early as the fourth week of deficiency, but more usually a little later—from the seventh to the twelfth week. As

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FIGURE 97. PYRIDOXINE DEFICIENCY.

Spinal cord, pig. A. Section through lumbar cord to show degeneration of dorsal columns B. and C. Dorsal and ventral roots, respectively, to show involvement of former and not of latter. D and E Higher power of dorsal and lateral columns to show involvement of former.

lipid accumulation may reach the mid-zonal regions Fatty livers have been observed in swine in the presence of adequate dietary choline and inositol

A slight increase (14 mm) in blood pressure has been noted in young and adult pyridoxine-deficient rats; no vascular changes have been found microscopically.[907]

In the B_6-deficient rat the thymus appears to show a marked reduction in size, greater than the atrophy which may be associated with a similar degree of manition[875] This relationship to lymphoid tissues has led to the study of antibody production in pyridoxine-deficient rats[753, 875] Agreement is general that the formation of antibodies to several types of antigens is impaired. For instance, antibody titers to sheep erythrocytes and the H antigen of B typhosum were lower in deficient rats than in controls[875] Total white blood cell counts, particularly lymphocytes, remained unchanged. These studies are of extreme interest in view of the important role of pyridoxine in protein metabolism.

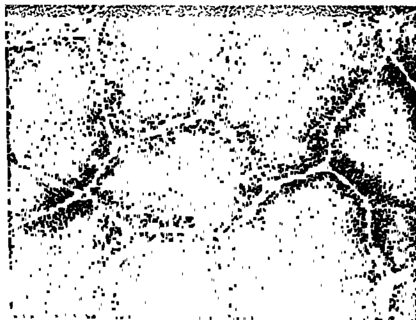


FIGURE 98 PYRIDOXINE DEFICIENCY

Liver, pig Section of liver from pig which had been on a pyridoxine deficient diet for 113 days and was then sacrificed The animal had developed severe anemia and convulsions, together with evidence of neurological involvement. There is extensive fatty infiltration of the central portions of the liver lobules and deposition of hemosiderin pigment about the peripheral portion, which accounts for the dark staining in this region The liver of the pig is, of course, normally lobulated Prussian blue fuesin stain (x 35).

many as three or four attacks per day have been observed; the "fits" may occur until death ensues, unless treatment with pyridoxine is initiated. Such convulsions resemble those seen in the "grand mal" of human epilepsy. Attacks of shorter duration, which resemble human "petit mal," have also been observed. Preceding the convulsion the animal is usually excited and "nervous." The pattern of the convulsion has been described as follows: "The pig lay on its side; all four limbs and the muscles of the body jerked rapidly, the head was held in extension, the eyes shut or turned upward, and saliva drooled from the mouth. After several minutes the spasmodic muscular contractions ceased, and a stage of stupor followed which also lasted several minutes. Occasionally a gurgling sound could be heard When the stupor was over, the pig would try to get up; and, when it finally succeeded, it would proceed in a staggering, dazed fashion."[936]

An ataxia, which has been observed in swine as early as the third week, manifests itself as a slightly high lift of the hind limbs accompanied by swaying of the hind quarters while walking. There is a broad base; the legs fold under, turning in one direction or another, with the result that the animal stumbles and falls The forelegs develop similar incoordinations so that as the deficiency progresses, the animal becomes completely incapacitated.

When the behavior during life is compared with the anatomical changes found at autopsy, it appears that physiological disturbances may be present before morphological alterations can be demonstrated.[912] The initial anatomical change appears to be demyelinization of the peripheral nerves (brachial and sciatic). This is characterized by the presence of small droplets of neutral fat in sections stained with Scharlach R, and by vacuoles and dark deposits in Weigert preparations. Alterations in anisotrophic properties are also seen. Silver stains for the demonstration of axis cylinders reveal only questionable degeneration of these structures at this stage. No alterations are detected in the cytoplasm of the dorsal-root ganglion cells. As time goes on, myelin degeneration becomes more marked peripherally and involvement of the dorsal-root fibers and even the dorsal columns of the spinal cord is found. Marked axonal degeneration accompanies these changes. Despite such alterations in the peripheral and central portions of the sensory neuron, no chromotolytic phenomena are encountered in the cell body. However, cells become atrophic and, in time, necrotic, but without the widespread dissolution of Nissl granules which one might expect and which is seen in pantothenic acid deficiency in swine.[885]

Convulsions have been noted in pyridoxine-deficient calves.[937]

By chemical analysis, an increased fat content of the liver of pyridoxine-deficient rats [938] and mice has been reported. A similar change has been observed histologically in swine [912] In the latter species the fat is distributed in the central areas, and in animals extremely deficient in pyridoxine

lipid accumulation may reach the mid-zonal regions. Fatty livers have been observed in swine in the presence of adequate dietary choline and inositol.

A slight increase (14 mm) in blood pressure has been noted in young and adult pyridoxine-deficient rats, no vascular changes have been found microscopically.[907]

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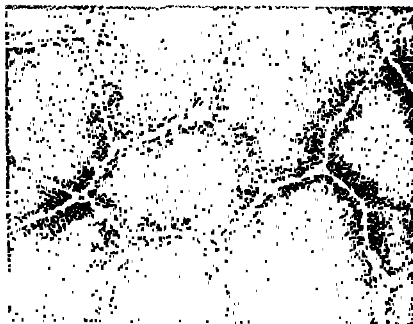


FIGURE 98 PYRIDOXINE DEFICIENCY

Liver, pig. Section of liver from [illegible]

[illegible] ...ation of the central portions of the liver lobules and deposition of hemosiderin pigment about the peripheral portion, which accounts for the dark staining in this region. The liver of the pig is, of course, normally lobulated. Prussian blue-fucsin stain (x 35).

An entirely unexpected and most interesting lesion has been described in pyridoxine-deficient Rhesus monkeys.[940, 941] The animals were observed from five to sixteen months. After several weeks they began to lose weight and appeared ill-kept. Some had edema of the eyelids. Later in the course of the experiment fissures appeared in the hands and feet. Pyridoxine and transaminase levels of the blood were reduced. At autopsy severe sclerosis was found in many arteries: coronary, renal, pancreatic, et cetera. The principal change consisted of fibrosis which separated the intima from the internal elastic lamella. Such changes as these are unique in nutritional deficiency disease Corn oil was the source of fat in these experiments. In addition to arterial changes, fatty livers, which go on to develop cirrhosis, have been observed. Moreover, another interesting abnormality, dental caries, has been described.[941]

Studies of the cornea in pyridoxine-deficient rats have revealed extensive vascular proliferation.[896] This is similar to that which has been described in deficiency of zinc,[233] amino acids,[920] riboflavin [831] and pantothenic acid.[876] Hence, it would not appear to be a specific alteration.

Reproduction has been specifically studied in female rats placed on pyridoxine-deficient diets with added desoxypyridoxine for varying periods of time.[942] There was a high incidence of resorptions which developed only after ten to twenty days on the deficient diet prior to breeding, a finding which distinguishes the effects of this deficiency from those of other vitamins on the reproductive process.

Three of four dogs deficient in pyridoxine have been reported to have developed signs of cardiac insufficiency and to have died suddenly.[930]

When adequate quantities of p-dimethylaminoazobenzene (butter yellow) are administered to rats, carcinoma of the liver develops. The incidence of tumor formation may be modified by diet. A reduction in the amount of dietary pyridoxine prevents the development of carcinoma. In an experiment such as this [943] a caloric effect can be ruled out, since the pyridoxine-deficient animals and their controls consume the same amounts of food. The effect of pyridoxine on sarcoma 180, a transplantable tumor in mice, has been studied; the removal of pyridoxine from the diet inhibits the growth of the tumor even though, as in experiments on rats, the caloric intake is the same.[944]

In the *human*, pyridoxine deficiency has been produced experimentally and has been observed to occur naturally.

Two infants, age two and eight months, have been maintained on a vitamin B_6-deficient diet for periods of approximately eighty and 160 days, respectively.[945] The younger infant developed a series of severe convulsions on the seventy-sixth day of deprivation of the vitamin. Improvement followed the intravenous administration of pyridoxine. The second

child gradually evidenced a microcytic anemia, by the 140th day of the deficiency hematologic findings were, Htc, 16 per cent; MCV, 64; MCH, 22, and MCHC, 34 per cent The administration of pyridoxine effected a striking remission with a reticulocyte rise up to 12 per cent. Studies of ability of these two infants to convert tryptophan to N-methylnicotinamid showed impairment in this metabolic transformation.

In 1954, several interesting reports[946,947] were published to call attention to a convulsive syndrome in infants which had been fed a liquid proprietary formula which had been autoclaved before use. The cause of the seizures was ascribed to pyridoxine deficiency Treatment cured the syndrome, the clinical improvement was immediately corroborated by electro-encephalographic tracings. Pyridoxine metabolism has been studied in children by measuring the excretion of xanthurenic acid and N-methylnicitonamide before and after a standard dl-tryptophan load test and by the estimation of the excretion of 4-pyridoxic acid and xanthurenic acid before and after parenteral pyridoxine plus a standard tryptophan load Some evidence of pyridoxine need was demonstrated

Pyridoxine deficiency has been studied by Vilter and his associates[948]



FIGURE 99 PYRIDOXINE DEFICIENCY

Human Seborrheic dermatitis about the nose from individual experimentally depleted of pyridoxine. (Courtesy of Dr. R. W. Vilter)

An entirely unexpected and most interesting lesion has been described in pyridoxine-deficient Rhesus monkeys.[940, 941] The animals were observed from five to sixteen months. After several weeks they began to lose weight and appeared ill-kept. Some had edema of the eyelids. Later in the course of the experiment fissures appeared in the hands and feet. Pyridoxine and transaminase levels of the blood were reduced. At autopsy severe sclerosis was found in many arteries: coronary, renal, pancreatic, et cetera The principal change consisted of fibrosis which separated the intima from the internal elastic lamella. Such changes as these are unique in nutritional deficiency disease. Corn oil was the source of fat in these experiments. In addition to arterial changes, fatty livers, which go on to develop cirrhosis, have been observed. Moreover, another interesting abnormality, dental caries, has been described.[941]

Studies of the cornea in pyridoxine-deficient rats have revealed extensive vascular proliferation.[896] This is similar to that which has been described in deficiency of zinc,[233] amino acids,[320] riboflavin [831] and pantothenic acid.[876] Hence, it would not appear to be a specific alteration.

Reproduction has been specifically studied in female rats placed on pyridoxine-deficient diets with added desoxypyridoxine for varying periods of time.[942] There was a high incidence of resorptions which developed only after ten to twenty days on the deficient diet prior to breeding, a finding which distinguishes the effects of this deficiency from those of other vitamins on the reproductive process.

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the metabolism of tryptophan, alanine, and urea It is of interest that the administration of cozymase relieved the glossitis and that high doses of linoleic acid improved the dermatitis. Pyridoxine cleared the skin lesions in areas where it was applied locally The human requirement for pyridoxine or its derivatives is estimated to be 2 to 3 μgm. per day.

Some indication of the state of vitamin B_6 concentration in the adult organism can be obtained from studies of the excretory pattern of tryptophan metabolites, in pregnancy, alterations may be observed.[949]

A compound, which is somewhat similar to pyridoxine, is isoniazid. A number of tuberculous patients treated therapeutically with this material have developed sensory neuritis. The administration of pyridoxine alleviates these unfavorable side effects[950]

Finally, vitamin B_6 deficiency may be concerned with anemia in the human The clinical course of an adult who had hematologic abnormalities which were unresponsive to usual therapeutic measures has been described. Hypochromic anemia, leukocytosis, high serum iron, and high per cent iron binding protein saturation were present. In addition, the metabolism of tryptophan was abnormal. Pyridoxine administration led to a reversion of these defects to normal[951]



FIGURE 100. PYRIDOXINE DEFICIENCY.
Human. Scaling dermatitis. (Courtesy of Dr R. W. Vilter.)

in man utilizing the anti-metabolite, desoxypyridoxine. Fifty individuals suffering from a variety of chronic diseases were given either a hospital diet or one low in B vitamin content; 50 to 400 mgm. of the antagonist were administered daily. Thirty-four of the group showed signs and symptoms of varying degree. The most striking was a "seborrheic dermatitis," characterized by erythema of the nasolabial folds which sometimes spread to involve the periocular tissues, eyebrows, and angles of the mouth. Itching and burning of the involved areas was noted The tongue, in particular, felt as if it had been scalded; the dorsum became reddened and resembled the glossitis of nicotinic acid deficiency. There was cheilosis, angular stomatitis, and some conjunctivitis, no keratitis was ever noted. One patient exhibited a sensory neuritis characterized by tingling and numbness of the feet, hyperesthesias, and impaired position and vibration sense. After therapy the feet became painful. Common subjective symptoms were anorexia, nausea and drowsiness A mild normochromic, hypoplastic anemia was observed in some and was accompanied by lymphocytopenia. As might have been expected from observations in animals, abnormalities were demonstrated in

CHOLINE

The nutritional importance of choline[952] first became apparent in 1930 when Hershey fed lecithin to depancreatized dogs which were being maintained on insulin.[953] The rationale for this procedure was to determine whether the fatty liver which was ordinarily encountered in such experimental animals[974] could be prevented by the administration of a phospholipid. Lecithin did just this. Furthermore, it prevented the fatty livers which resulted from the feeding of diets of high fat content to rats.[954] The active principle of lecithin was soon demonstrated to be choline.[955]

The relationship of choline deprivation to hepatic damage was soon demonstrated in several laboratories. At the same time Du Vigneaud had clarified the interrelationships of choline, methionine, and cystine, as well as the phenomenon of transmethylation.[957]

Another area affected by choline deficiency is the kidney, in which changes were first described by Griffith and Wade[956] in 1939.

Choline is, of course, an important constituent of the phospholipid, lecithin. The metabolism of choline is intimately related to that of the indispensable sulfur-containing amino acid, methionine. Although, even when dietary choline is absent, sufficient quantities may be formed from methionine *in vivo* to insure life,[657] not enough is synthesized to prevent certain physiological and pathological alterations in the animal organism. Choline is formed *in vivo* from the combination of ethanolamine and methyl groups donated by methionine;[958] ethanolamine is derived from dietary serine and glycine.[959]

The precise role of choline in metabolic processes has not been entirely worked out. At the present time two main effects have been demonstrated. The first is based on its relation to phospholipid formation and turnover. Such a role would seem to be extremely important since it has been assumed that fatty acids must leave the liver as phospholipids. When choline is omitted from the diet, phospholipid turnover is reduced in other tissues, for instance, radioactive phosphorus (P^{32}) may be utilized to show that choline stimulates phospholipid turnover in the kidney.[960] Choline enhances the transportation of fatty acids from the liver to the fat depots, a process which is slowed down in choline-deficient animals.[961]

Perhaps an even more important role for choline is based on its influence on fatty acid oxidation in the liver.[962] It has been shown that the oxidation of long chain fatty acids is depressed *in vitro* when liver preparations from choline-deficient animals are studied. If choline is administered *in vivo*, the ability of the liver tissue to oxidize fatty acids is restored. It must be

time goes on this lipoid infiltration extends out to involve the midzonal portions and thence to the periportal parts of the lobule. The discrete droplets of fat in time coalesce, so that each cell comes to contain one large globule of neutral fat-staining material At this stage, as Hartroft has shown,[975, 976] several cells, engorged with fat, may coalesce to form cyst-like structures which may rupture into or, at least, come into communication with the extracellular space, and even with the bile canaliculi or blood sinusoids Via the latter route emboli of fat may reach the heart, lungs or kidneys This pattern of deposition of fat in the central cells of the lobule where it begins as small droplets and the extension to involve the entire liver lobule, while the droplets coalesce to form large vacuolated cells, is the hallmark of choline deficiency in the experimental animal placed on a low protein and high fat regimen

How may this picture be modified, what is the chemical nature of the lipid, what is the pathogenesis of such fatty infiltration and, finally, what may be the outcome?

Modifications in the degree of fatty change may be brought about by altering the protein content of the diet Since methionine gives rise to choline, one must, if he wishes to produce choline deficiency, have as little methionine present in the diet as possible However, the fatty change is dependent on growth, hence, all other amino acids, particularly cystine must be present [977, 978] Threonine, tryptophan, and lysine appear to have some lipotrophic activity (page 105) So does vitamin B_{12} [1079] Of extreme importance is the kind of lipid present in the diet, i e, whether animal or vegetable, the degree of unsaturation, and the length of the fatty acid chains Butter fat exerts a greater need for choline than a similar amount of corn oil [979] The saturated fatty acids are more conducive to the production of fatty livers than those which have one or more unsaturated bonds Moreover, the even numbered fatty acids containing twelve or less carbons do not give rise to fatty liver when they are incorporated into a choline-deficient diet On the other hand myristic (C-14), palmitic (C-16) and stearic (C-18) acids do lead to fatty livers, though in decreasing degree.[980] Cholesterol feeding increases and alters the pattern of fat deposited in the liver.[981, 983] Fatty infiltration may be produced on a low fat diet, although the lipid may take longer to appear [983]

Another way in which the accumulation of fat in the liver may be modified is by experimentally affecting thyroid activity When "anti-thyroid" substances, such as thiouracil, sulfaguanidine or para-aminobenzoic acid, are administered to rats on a choline-deficient regimen, less fat accumulates in the liver cells. On the other hand if thyroid extract is fed, excessive fatty infiltration ensues.[984]

The fat content of the normal or diseased liver may be expressed in vari-



FIGURE 101. CHOLINE DEFICIENCY

Liver, rat *A* Early accumulation of fat about central vein *B*. More diffuse involvement to show variations in involvement of the cells H. and E. (× 100).

postulated, of course, that choline stimulates or participates in the formation of active substances formed *in vivo.*

The most striking and extensively studied alteration resulting from choline deficiency is found in the liver, where massive fatty infiltration occurs This change has been observed in rats,[957, 963, 964, 965, 966] mice,[967] rabbits,[968] guinea pigs,[969] hamsters,[970] dogs,[971] calves,[972] and swine.[973] The most suitable diet with which to demonstrate the development of fatty liver is one low in methionine, such as 8 to 10 per cent casein (which may be supplemented with cystine), and high in fat (15 to 20 per cent), together with adequate minerals and all vitamins save choline. Certain ways in which the hepatic alterations may be reduced or aggravated will be mentioned below

The initial change in the liver may appear early, sometimes after two days. Small globules of neutral fat are seen in the cytoplasm of the cells of the central portion of the liver lobule, i e, those about the central vein As

fall back on the two best known, though not as yet, too well-understood metabolic functions of choline on the liver: to promote phospholipid turnover and to enhance oxidation of fatty acids By these two mechanisms the liver cells are normally able to clear themselves of fatty acids which are brought to them by the blood stream, whether from ingested lipids or from the breakdown of fat elsewhere, particularly in the depots of the subcutaneous tissues and other areas.

Studies of the biochemical activities of the liver in choline–deficient animals are of interest.[986, 987] During the early stages of fatty infiltration, chemical evidences of liver cell damage have been demonstrated. Such changes consist of bromsulphthalein retention and increased concentrations of bilirubin in the serum Choline supplementation corrects these alterations. However, such therapy fails to ameliorate other evidences of hepatic damage, such as elevation of serum alkaline phosphatase activity and reduction of serum esterase. Though such livers show no alterations morphologically it must be assumed that the low protein diet utilized to study choline deficiency leads to physiological alterations in the liver cells, possibly related to the necrogenic factors discussed on page 98

In puppies, choline deficiency may result in death within three weeks.[971, 988, 989] Severe fatty infiltration of the liver is the only prominent manifestation of this deficient state, livers from such animals may contain over 50 per cent of lipid on a dry weight basis, this is over twice that of control animals When bromsulfalein is administered, the dye remains in the plasma longer than normal. So, too, the prothrombin time is increased, and the level of serum alkaline phosphatase rises These manifestations of deranged liver function can be reversed in five to ten days if choline is administered in adequate amounts[989]

Morphologic observations of the hepatic changes which follow choline therapy have been reported[986, 987, 990] In the rat the color of the organ changes from yellow to dark reddish brown, and the size decreases On microscopic section a reduction in the amount of fat is observed in the cells, in addition, some evidence of regeneration of hepatic cells is found with large, bizarre structures containing several nuclei

Thus far we have purposely made it appear as though the primary and only effect of choline deficiency on the liver was to promote extreme fatty infiltration Studies of such fatty livers which have been allowed to persist for prolonged periods have revealed that scarring is a prominent feature. Hence, such scarring, or cirrhosis, was interpreted by many to have a direct causal relationship Early studies of cirrhosis in rabbits,[968] rats,[961, 965, 966, 973] and dogs[988] clearly appeared to indicate that fatty change led to scarring.

When one studies the livers of rats which have been subsisting on lipogenic diets for prolonged periods, the earliest evidence of new connective

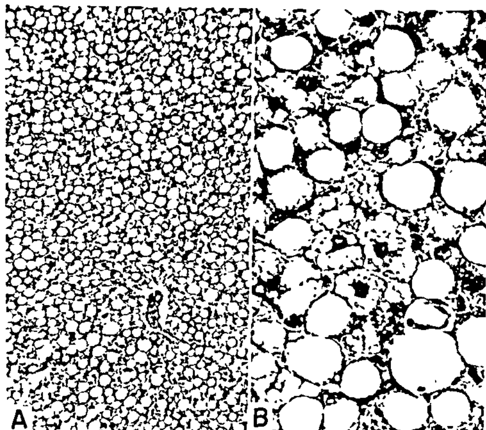


FIGURE 102. CHOLINE DEFICIENCY.

Liver, rat. Diffuse involvement of the entire organ is seen in *A* (x 100). Most of the cells under higher magnification, *B* (x 450) are distended with fat, in a few small vacuoles are present H and E.

ous ways, the two most common methods of evaluation are to formulate total hepatic lipid on a wet or dry weight basis. The normal rat liver will be *found to contain 6 3 and 22.7 per cent total fat, respectively.*[985] *Livers from* choline-deficient rats may contain up to 40 and 70 per cent total lipid when either of these two methods of expression is used. Alterations in the chemical composition of the liver have been studied. As might be expected there is a marked increase in hepatic weight after three weeks on a hypolipotrophic diet, from 5.15 grams for a group of 100-120 gram animals up to 9.2 grams. The percentage increase in the amount of total new material appearing is as follows: dry fat free tissue, 12.4; water, 40 6, glycerides, 46 0, cholesterol, plus ester, 0.8; and phospholipids 0 2.[985] Various levels of cholesterol in the diet affect the relative amounts of these constituents.

As for the pathogenesis of the fat accumulation in the liver one must

fatty cysts and the condensation of their remnants as the precursor of the fibrous change.[976] The problem of the pathogenesis of experimental cirrhosis is a knotty one Unfortunately, we shall have to confront it again when nutritional disease of the liver in man is discussed (page 344).

The role of intestinal bacteria in the pathogenesis of experimental cirrhosis in rats has assumed great importance as a result of recent data reported by Ruttenburg *et al.*[1505] They placed rats on a diet of choline-free peanut meal and casein, lard, minerals and vitamins. To the diets were added absorbable or non-absorbable antibiotics. The results were dramatic. In the control group 80 per cent (24 of 27 animals) developed cirrhosis after 300 days, 73 per cent (8 of 11) of the rats given absorbable antibiotics (tetracycline) with the choline-deficient diets showed cirrhosis when killed after 400 or more days Only 17 per cent (3 of 18 rats) given non-absorbable

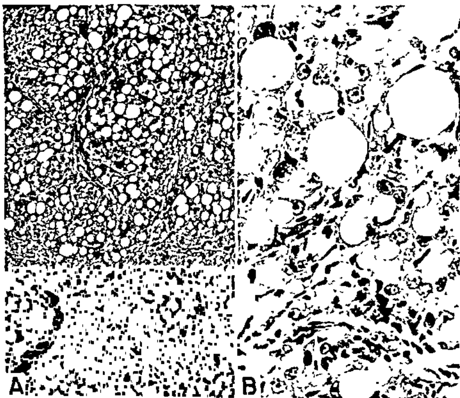


FIGURE 104 CHOLINE DEFICIENCY

Liver, rat *A* Diffuse scarring with separation of parenchyma into lobules most of whose cells contain fat (x 150). *B* Higher power to show great variation in size of fat-containing cells (x 450) H and E.

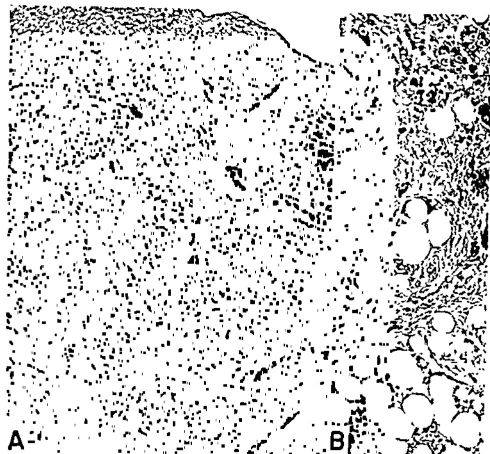


FIGURE 103. CHOLINE DEFICIENCY

Liver, rat. Chronic deficiency with cirrhosis. *A* The tissue is broken up into lobules of varying sizes Note some contain fat while others do not (x 100). *B* Higher power (x 250) shows fatty globules encased by connective tissue. Masson connective tissue stain

tissue is found about the central vein.[991] Here, argyrophilic fibers of reticulum are found in greater numbers than normal. They also come to extend out farther and farther into the lobule and in time reach the periportal areas. What is the cause of such scarring? Disturbed sinusoidal blood flow in the central portion of the lobule because of the central fatty infiltration has been accused.[991] If there is some collapse or necrosis of central liver cells this process may be accentuated. It has further been suggested that choline deficiency may injure the liver cell to the extent that it stops its normal regenerative pattern [992] Scarring could then be looked upon as an ordinary reparative phenomenon. Another concept looks upon the formation of

terial, ceroid has basophilic properties, gives a *negative iron reaction*, is not dissolved by lipid solvents, and exhibits a positive oxidase reaction. Unlike vitamin A, its fluorescence does not fade when tissue sections are viewed under ultraviolet light[996] A number of investigators have studied the production and properties of ceroid, thinking it might be related in some way to the development of hepatic damage It is now clear, however, that by a modification of the diet, cirrhosis may be produced without a concomitant deposition of ceroid.[997] Today, there is general agreement that ceroid is a metabolic artifact, which results from the presence of excessive amounts of cod liver oil or other unsaturated lipids in the diet

Renal lesions, first described in rats by Griffith and Wade[95b] have now been extensively studied by other investigators[998, 999, 1000, 1001, 1002] When young rats are placed on the usual low-protein, choline-deficient diet, gross evidence of damage to the kidneys appears in four or five days The organs are enlarged bilaterally By the ninth or tenth day they have become ex-



FIGURE 106 CHOLINE DEFICIENCY

Kidney, rat *A* Section through cortex which shows disorganization of tubular structures and the thickening of capsule (x 50) *B* Higher power (x 200) which shows necrosis of tubular epithelial cells, and red blood cells in lumens of the tubules

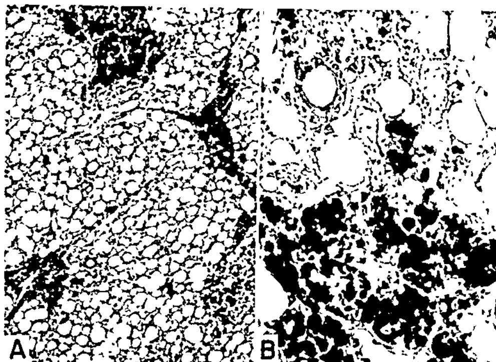


FIGURE 105. CHOLINE DEFICIENCY

Liver, rat. *Ceroid deposition Two fields, A (x 100), and B (x 450), to show deposition* of pigment in macrophages and between liver cells. Acid fast stain.

antibiotics and killed from the 400th to 750th day showed cirrhosis Fatty infiltration was present in all groups. If such experiments as these can be readily repeated, they would appear to go a long way toward providing an answer to the knotty problem of the pathogenesis of cirrhosis in choline-deficient fatty livers and would, of course, offer a therapeutic approach to cirrhosis in man

A number of studies have been concerned with the healing sequences which follow the administration of choline, protein or methionine.[990, 993, 994, 995] It appears that protein feeding is more effective in inducing reparative phenomena than is the administration of choline or methionine alone. The favorableness of the responses is clearly related to the severity of the deficient state at the time therapy is instituted.

During the development of our knowledge of the relationship which choline and the sulphur-containing amino acids have to one another and to morphological changes in the liver, several groups of investigators described and studied a peculiar pigment which occurs in and about the hepatic cells of rats on choline-deficient diets. Because of its waxy appearance such pigment was named "ceroid."[995] An acid-fast, hyaline-like ma-

which have an important bearing on the outcome have been studied.[1003] Besides the amount of methionine in the ration, the cystine and fat contents of the diet are important. Cystine has been noted to have a deleterious effect on choline-deficient animals.[977, 978] The reason is not entirely clear, although it has been suggested that the amino acid promotes better growth and hence an increased requirement for choline. Because of the relationship of choline-containing phospholipids to fat transport, high-fat diets and variations in the degree of saturation of the fat may have a devastating effect on choline-deficient animals.[943] The protein content of the diet is another important factor when the cystine and fat content do not vary.[1003] Protein (casein) levels of 15 per cent are optimal for the production of hemorrhagic changes in the kidney; when dietary protein is reduced, hemorrhages appear less readily. However, at a protein level of 6 per cent renal lesions do not appear in the usual time, but may take forty to fifty days to present themselves. The type of dietary carbohydrate is also of importance since lesions develop when sucrose, glucose and starch are the source of carbohydrate in the diet, while when lactose and galactose are substituted the kidneys remain normal.[1084]

Renal lesions have been mentioned in swine[973] and calves,[972] although in these species they have not been thoroughly studied. In other species, in which fatty livers are readily produced, renal changes are not conspicuous.

The renal lesions in rats are apparently responsible for hypertension which has been described in this species. Rather extreme alterations have also been found in the heart and blood vessels, and in the myocardium.[1004, 1005, 1006] The pathogenesis of these lesions in the heart is not clear. They consist of focal areas of necrosis which may then be replaced by connective tissue. The relation of choline to cardiovascular lesions produced by large amounts of vitamin D has been studied.[1006]

Certain other manifestations of choline deficiency must be noted. Rats develop anemia and edema. The latter is related to a decrease in plasma protein concentrations. This may be raised if choline is administered.[1007, 1008] In addition to the disturbances in plasma protein values liver disease also undoubtedly plays a role. Anemia and edema has also been described in dogs.[1009]

Muscular dystrophy has been reported in choline-deficient rabbits.[1010, 1011] This change resembles the lesions encountered in vitamin E deficiency. Further study is certainly indicated.

Finally, neoplasms have been observed in the lungs, liver, and other tissues of choline-deficient rats.[1087] The pulmonary tissues resemble adenocarcinomas. Solid hepatoma-like neoplasms are found in the liver in association with cirrhosis. Other hepatic tumors resemble adenocarcinomas. Several different types of connective tissue tumors are described in other areas.

tremely large, comprising almost 3 per cent of the total body weight. As they begin to enlarge the external surface becomes congested, hemorrhages appear beneath the capsule. After the maximum increase in size the organs gradually decrease so as to reach normal proportions after a few weeks.

Microscopically, the initial alteration is seen by the third or fourth day. Small droplets of fat appear in the cytoplasm of the cells of the proximal tubules. These increase in number and size so as to increase the total dimensions of each cell. In severely affected kidneys many necrotic tubular cells are seen. The development of necrosis appears to coincide with evidence of stasis, i.e., inter-tubular engorgement and frank hemorrhage. No red cells are seen in the tubules themselves, though casts which give some of the staining reactions for hemoglobin derivatives are present in collecting, as well as the proximal and distal segments. The glomeruli appear unaffected So, too, the blood vessels are not involved. Specific examination of the tubular epithelial cells reveals a decrease in mitochondria.

As might be expected, certain physiological and biochemical alterations result from these anatomic changes. Oliguria develops and is accompanied by nitrogen retention. Decreases in the excretion of sodium, potassium and creatinine are found, hence, electrolyte levels rise in the serum.

How are the anatomic alterations to be explained? The initial change appears to be an accumulation of fat in the tubular epithelial cells. This fatty infiltration may represent a decreased ability for the cell to turn over fat just as is occurring in the liver at the same time. Phospholipid metabolism, as measured by P^{32}, is decreased in the choline-deficient kidney (page 251), so, too, oxygen consumption is reduced. The most conspicuous change is vascular stasis, followed by hemorrhage. Such circulatory derangements appear to be mechanical and are brought on by enlargement of the cells which make up the tubules. Vascular deficiency together with fatty infiltration are both responsible for the cellular necrosis already mentioned. An attempt has been made to study the distribution of activities of alkaline and acid phosphatases.[1001, 1002] A decrease in the former enzyme appears after well-marked necrosis of the tubular epithelial cells has occurred.

The severity of the damage apparently determines whether an animal will or will not recover, since many rats do not succumb, even though continued on the choline-deficient diet. In such animals the tubular epithelium regenerates to a low cuboidal type; calcification also occurs and many of the tubules become dilated. When large areas of necrosis have resulted, scars may be observed. In such recovered organs connective tissue proliferation in the capsule is noted, so that grossly the organs have a "frosted" appearance.

Attempts to produce renal changes by means of choline deficiency, in the rat at least, do not yield entirely consistent results. Some of the factors

which have an important bearing on the outcome have been studied.[1003] Besides the amount of methionine in the ration, the cystine and fat contents of the diet are important. Cystine has been noted to have a deleterious effect on choline-deficient animals.[977, 978] The reason is not entirely clear, although it has been suggested that the amino acid promotes better growth and hence an increased requirement for choline. Because of the relationship of choline-containing phospholipids to fat transport, high-fat diets and variations in the degree of saturation of the fat may have a devastating effect on choline-deficient animals.[983] The protein content of the diet is another important factor when the cystine and fat content do not vary.[1003] Protein (casein) levels of 15 per cent are optimal for the production of hemorrhagic changes in the kidney; when dietary protein is reduced, hemorrhages appear less readily. However, at a protein level of 6 per cent renal lesions do not appear in the usual time, but may take forty to fifty days to present themselves. The type of dietary carbohydrate is also of importance since lesions develop when sucrose, glucose and starch are the source of carbohydrate in the diet, while when lactose and galactose are substituted the kidneys remain normal.[1084]

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BIOTIN

Present knowledge of biotin developed along three independent lines of research. From the beginning of the century on, a number of investigators had called attention to the deleterious effects which follow the feeding of unheated egg white to experimental animals. Boas[1012] postulated in 1927 that egg white contains a "toxic" substance, which is rendered innocuous by the inclusion of certain food substances containing a protective "x factor" in the diet. Rats fed egg white develop dermatitis, an abnormal kangaroo-like posture, and spasticity of the extremities. During the ten years following Boas' publication, a number of investigators studied the syndrome of egg white injury. Gyorgy,[1013] in particular, described the histological changes in the skin and demonstrated the presence of a curative material in certain foodstuffs; this factor was designated "vitamin H." In the meantime, workers in other fields were providing information which was to clarify the problem. A new factor named coenzyme R had been described as an essential for legume nodule bacteria in 1933.[1014] A little later a crystalline material, Bios II, was shown to be necessary for the growth of yeast cells.[1015] In 1939, the suggestion was made that coenzyme R and Bios II were identical[1016] and a year later du Vigneaud and his associates[957] proved that Gyorgy's vitamin H, coenzyme R and Bios II were one and the same substance. In 1942, du Vigneaud[957] announced the structure of biotin; an active substance was soon synthesized and found to elicit the same physiological responses as the natural product. While the structure of this vitamin was being elucidated an active anti-biotin principle from egg white was crystallized; this material is called avidin.[1017, 1018]

One of the important functions of biotin appears to be its role in carbon dioxide fixation. Here it is part of a coenzyme which affects such reactions as the decarboxylation of oxalacetate and the synthesis of citrulline from ornithine.[1019]

Biotin has been shown to be a dietary essential for the rat,[1020, 1021] mouse,[1022, 1023] hamster,[1024] rabbit,[1025] dog,[1026] calf,[1027] pig[1028] and the monkey.[1029] Skin lesions have been described in several of these species; in addition, studies of the nervous tissues and muscles have been made. The role of biotin in tumor formation has also been investigated.

When young rats are placed on a diet containing 30 per cent dried egg white, gross and microscopic cutaneous lesions develop after three to five weeks.[1020, 1021] The initial change is a generalized erythema, the coat becomes roughened and loses its luster. Widespread scaling then follows, which is accompanied by a symmetrical alopecia, first developing over the



FIGURE 107. BIOTIN DEFICIENCY

Skin, rat A External appearance of a rat which had been placed on a diet containing 30 per cent egg white. There is complete absence of hair and the entire body is covered by greasy yellow scales Note also the humped position and gait B Skin from late stage similar to that depicted grossly [illegible] of the orifices of the hair follicles This results in [illegible]
There is relatively little change in [illegible]
Sullivan and the *Archives of Dermatology and Syphilology*)

chin, neck, and anterior portion of the venter and spreading to the rest of the body surface. Such rats, which are covered with brown, greasy scales, are not particularly pleasant sights.

Microscopically, the skin shows a hyperkeratosis, together with a uniform acanthosis or increase in the prickle cell layer. A sparse, but definite, diffuse cellular infiltration of the corium then appears; the collagenous fibers are spread apart by edema fluid. The shafts of the hair follicles become

dilated and their patulous orifices are clogged with hyperkeratotic sudanophilic material As the late stage of the disease is reached, the epidermis has become atrophic. The sebaceous glands diminish in size and the epidermis exhibits small superficial ulcers The meibomian glands are not affected. Biotin concentrates completely ameliorate these cutaneous changes

Biotin-deficient hamsters [1024] develop a dermatitis at the corners of the mouth, such lesions spread more excessively as the deficiency progresses Alopecia occurs in biotin-deficient mice [1022] [1023] The hair of black biotin-deficient mice loses its color and falls out [1024] Dermatitis and alopecia have been described in rabbits fed a diet containing excessive amounts of egg white.[1025] In swine [1028] the dermatitis and alopecia are prominent when egg white is fed Such changes are followed by cracking and bleeding of the soles and tops of the hoof heads. Inflammation of the mucous membranes of the mouth is prominent In calves [1027] inhibition of growth and paralysis of the hind quarters have been described A scaling dermatitis has been described in monkeys [1029]

In mice biotin appears to be a chromotrichia factor, for when black coated animals are placed on a deficient regimen their hair first becomes rusty, then gray The administration of biotin to such animals leads to the resumption of normal color of newly formed hairs.[1023]

The peculiar attitude and gait of biotin-deficient rats have prompted a study of the nervous tissues and muscles of these animals [1021] Careful examinations of forebrain, hind brain, spinal cord, posterior root ganglia and sciatic nerve have failed to reveal any abnormality On the other hand, studies of muscle tissues have demonstrated atrophy, necrosis of fibers, and an increase in sarcolemma nuclei, all of which are similar to the changes which are observed in alpha-tocopherol deficiency This observation has prompted the addition of large doses of vitamin E to the diet of a second group of biotin-deficient animals, only atrophy was found in the muscles at autopsy It appears that biotin is not the etiological factor responsible for the changes described in the first group of animals. A single myographic reading following stimulation of the sciatic nerve gave no evidence of repetitive discharge, this would indicate that the rigidity which is observed is not myotonic in origin In view of the physiological changes which appear in the muscle, it is of interest to note that higher creatine contents of sciatic muscle from deficient animals have been noted than in controls; it is unfortunate, however, that comparison was not made with inanition controls Studies of other tissues of biotin-deficient rats have not revealed any other significant morphological changes, some evidence has been presented that biotin deficiency leads to an anemia in dogs [1026]

When biotin is added to diets which ordinarily prevent rats from developing hepatic tumors produced by butter yellow, a decrease in the protec-



FIGURE 107 BIOTIN DEFICIENCY.

Skin, rat *A* External appearance of a rat which had been placed on a diet containing 30 per cent egg white. There is complete absence of hair and the entire body is covered by greasy yellow scales Note also the humped position and gait. *B.* Skin from late stage similar to that depicted grossly. Note hyperkeratosis with dilatation of the orifices of the hair follicles This results in a peculiar finger-like appearance of the epithelium There is relatively little change in the underlying corium (Courtesy of Dr. Maurice Sullivan and the *Archives of Dermatology and Syphilology*)

chin, neck, and anterior portion of the venter and spreading to the rest of the body surface. Such rats, which are covered with brown, greasy scales, are not particularly pleasant sights.

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INOSITOL

Although inositol was isolated from living tissues during the last century, its designation as an essential nutrient did not come until 1940 when Woolley[1033] described alopecia in mice which had been placed on a diet deficient in this substance Since this report, positive and negative experimental results have been recorded At the present time, however, the essential nature of dietary inositol seems established The variations in biological response, which have been so confusing, appear to have been due to differences in bacterial synthesis of inositol by the gastrointestinal flora

Inositol is a constituent of a phosphatide derived from brain and as such may function in fashion similar to choline[1034] A relationship to fat metabolism is shown in its action to prevent fatty infiltration in the liver.[1035, 1036] On the other hand, it appears to enhance the incidence and severity of renal lesions on a choline-deficient and low protein diet.[1003] Beef heart is a rich source of inositol, containing 1 6 per cent on a dry weight basis.[1037] The significance of this remains to be determined

No histological studies have been reported on animals depleted of inositol Positive effects on growth have been reported in mice,[1033] rats,[1038]



Figure 108 Inositol Deficiency

Skin, mouse Animal which had been placed on an inositol-deficient diet. Note loss of hair over body with fur still remaining over head and extremities. (Courtesy of Dr. D W Woolley)

tive value of the diet occurs.[1030] In other words, biotin appears to act as an anti-inhibitor of the growth of this type of neoplasm in rats As might be expected, this finding has led to the therapy of human cancer with avidin but this far the administration of egg white or avidin to humans with malignant tumors has not met with encouraging results.

Biotin deficiency has been studied in four human subjects to whom 200 grams of dehydrated egg-white were fed daily.[1031] After three to four weeks on such a diet, all four volunteers developed a fine, non-puritic scaling of the skin. Although this dietary regimen was maintained, the skin lesions disappeared after a time. One subject developed a muculosquamous dermatitis of the hands, arms, and legs All evinced a peculiar grayish color of the skin and all ultimately showed atrophy of the lingual papillae. Anorexia, extreme lassitude, sleeplessness, and muscle pain also accompanied the deficient state. Two subjects complained of precordial distress; electrocardiographic alterations were present. Biotin therapy afforded prompt relief of these signs and symptoms. Spontaneous biotin deficiency in man seems extremely remote, since balance studies indicate that sufficient amounts of the vitamin are synthesized by the intestinal flora and are absorbed in such large quantities that an exogenous source of the vitamin is not necessary.[1032] Moreover, it is unlikely that the ordinary diet could ever contain enough of the anti-biotin, avidin, to lead to biotin deficiency.

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No histological studies have been reported on animals depleted of inositol. Positive effects on growth have been reported in mice,[1033] rats,[1038]



FIGURE 108. INOSITOL DEFICIENCY

Skin, mouse. Animal which had been placed on an inositol-deficient diet. Note loss of hair over body with fur still remaining over head and extremities. (Courtesy of Dr. D. W. Woolley.)

cotton rats,[878] hamsters,[1024] and strains of human cells in tissue culture.[1479]

Inositol has been shown to decrease liver fat ordinarily found in *human* patients with gastrointestinal carcinomata.[1039] This is the *only* observation reported on this material in man.

PARA-AMINOBENZOIC ACID

Evidence that para-aminobenzoic acid is an indispensable nutrient was presented by Ansbacher[1040] in 1941 The exact role of para-aminobenzoic acid in cellular metabolism is not clear save for its presence in the pteroylglutamates.[1050] It is of interest that PABA stimulates folacin production by certain types of bacteria PABA is an anti-thyroid compound[244] though its precise mechanism of action in this regard has not been elucidated

Ansbacher[1040] has been able to produce achromotrichia in rats on a synthetic diet and to restore the color of the fur with para-aminobenzoic acid. The achromotrichia is apparently unrelated to pantothenic acid or copper deficiencies (pages 233 and 62). No microscopic studies have as yet been reported on the tissues of para-aminobenzoic acid-deficient animals



FIGURE 109 PARA-AMINOBENZOIC ACID DEFICIENCY

Hair rat A Rat which had been on a para-aminobenzoic acid-deficient diet for four weeks B Rat on same deficient diet for four weeks and then two weeks on the same diet supplemented with 3 mg. of para-aminobenzoic acid per day. (Courtesy of Dr. S Ansbacher.)

FOLACIN AND FOLINIC ACID

The first experimental production of experimental macrocytic anemia was reported by Wills in 1932.[1041] Impressed by the prevalence of macrocytic anemia in the natives of Bombay, India, and because the anemia could be treated successfully with yeast concentrates, Wills placed monkeys on a dietary similar to that partaken by the natives (polished rice, white bread, wheat chapatti, ghi, white pumpkin, and small amounts of meat). The experimental animals developed a severe macrocytic anemia.

In 1935, Day and his associates[1042] described a syndrome in monkeys which consisted of anemia, leukopenia, necrosis of the gums, and diarrhea, these signs developed on a diet deficient in the vitamin B-group other than thiamine. Further studies eliminated riboflavin and nicotinic acid as possible causal factors of the changes exhibited by these monkeys,[1043] an uncharacterized substance present in liver and yeast was finally designated as the active principle or vitamin M. Doan and his collaborators[1044] then showed that a similar anemia and leukopenia in the monkey could not be cured by the addition of pyridoxine and calcium pantothenate to the other crystalline vitamins in the diet, but that the anemia could be prevented by a concentrate of material derived from leafy vegetables (folic acid). A little later a new material called *L. casei* Factor (see below) was found to be effective in curing the anemia of deficient monkeys.[1045]

While these investigations on primates were being carried out, Daft and Sebrell,[1046] had shown that "folic acid" cured a granulocytopenia which developed in rats whose diets contained sulfaguanidine, included to reduce the intestinal flora. During this period, the factors referred to above had been isolated from various sources and had been shown to be indispensable for the growth of certain micro-organisms, such as *Lactobacillus casei* and *Streptococcus fecalis*, hence the term, "*L. casei* Factor." Others had called a similar active compound derived from leafy vegetables, "*folic acid*".[1047] The complicated story of this era has been reviewed elsewhere.[1048] For several years the nature and interrelationships of these materials were not entirely clear. However, in 1945 the synthesis of an active *L. casei* factor was reported[1049] and during the latter part of that year and the early months of 1946 a number of clinical reports appeared, all of which demonstrated the importance of this new compound in the treatment of various types of macrocytic anemia in the human. The structure of this synthetic material, pteroylglutamic acid, was finally announced in 1946[1050] to consist of three substances: a two-ringed nitrogenous compound (a pteridine), para-aminobenzoic acid and glutamic acid. The latter occurs in varying

amounts depending on the natural source from which the active compound is derived. At the present time three pteroylglutamates are recognized: the monoglutamate (folic acid or folacin), the triglutamate (fermentation factor) and the heptaglutamate (vitamin B_c conjugate). In 1949, the American Institute of Nutrition adopted the name "Folacin" as a synonym for "folic acid."

The active form of folacin appears to be reduced folacin or folinic acid (also called citrovorum factor).[1050] Exactly how this transformation is brought about is not entirely clear, though it has been claimed that ascorbic acid is concerned in the conversion.[1051] The functions already ascribed to folacin would appear to apply equally well to folinic acid. At the present time several physiological aspects are being actively investigated: the metabolism of the so-called 1-carbon fragment, formate (HC=O), the metabolism of tyrosine, and interrelationships with ascorbic acid.

Folacin deficiency leads to a variety of disturbances in the handling of 1-carbon compounds, such as methyl synthesis and transfer and the synthesis of serine, purines and thymine.[1052] The nature of an active formyl compound analogous to acetylcoenzyme A has not been entirely elucidated though there is some evidence that folinic acid may serve as the coenzyme of transformylation. Folacin deficiency has been shown to lead to an accelerated conversion of methyl to formate and a decreased synthesis of formate from glycine.[1053] Folacin-deficient rats cannot methylate nicotonamide efficiently; administration of folacin leads to a marked increase in urinary excretion of methyl-nicotinamide.[1054]

Like ascorbic acid (page 176) folacin appears to be concerned with the metabolism of tyrosine If ascorbic acid-deficient guinea pigs, in which increased amounts of hydroxphenyl acids are found in the urine, are given folacin, the abnormal excretions of such compounds are stopped.[1055] A similar suppression has been demonstrated in scorbutic infants, though extremely large amounts of folacin must be administered to elicit the response.[1056] Folacin has no action on the excretion of tyrosine products in the scorbutic monkey.[1057]

Another relationship between folacin and ascorbic acid is concerned with red blood cell formation. When monkeys are placed on a milk diet deficient in ascorbic acid, scurvy develops accompanied by a megaloblastic anemia.[1058] Monkeys fed the same milk diet supplemented with adequate amounts of ascorbic acid maintain normal blood counts and a normal bone marrow picture. Either folacin, folinic acid or ascorbic acid will cure the blood changes, vitamin B_{12} will not. Further studies on monkeys[1059] have led to the conclusion that in scurvy the metabolism of folic acid is not deranged, but apparently the disease syndrome leads to an increased requirement for folacin over that contained in the milk diet which was employed.

These experiments are of interest in relation to the megaloblastic anemia of infancy to be discussed on page 435.

The effectiveness of anti-folacin compounds in studying folacin deficiency must also be mentioned. Such antagonists as 4-amino-pteroylglutamic acid (aminopterin) and 4-amino-10-methylpteroyl glutamic acid (A-methopterin) have yielded valuable information Folinic acid will reverse the untoward effects of these materials [1060]

The pteroylglutamates have been shown to be dietary essentials for the rat,[1046] guinea pig,[1062] dog,[1044] lamb,[1067] cat,[1063] mink,[1087] pig [1064, 1065, 1066] and monkey [1058 1059] As might be expected from the historical development of our knowledge of these compounds, most attention has been given to the blood forming tissues. Other effects will be discussed below

As mentioned above, the first experimental production of macrocytic anemia in monkeys was reported by Wills *et al* [1041] The studies of Day [1043, 1045] helped very little to characterize the blood dyscrasia which appeared in monkeys The most extensive studies in this species have been reported by May and his collaborators,[1058 1059] particularly with respect to the dual role of ascorbic acid. When animals are placed on a diet of milk without added ascorbic acid and folacin, a macrocytic anemia and leukopenia occur. The bone marrow is megaloblastic. Such changes are relieved by large amounts of folacin or folinic acid but not by ascorbic acid The final conclusion of these workers is that the "stress" of ascorbic acid deficiency increases the organism's need for folacin and, in order to prevent the hematological changes, increased amounts of the pteroylglutamate must be administered. Pathological changes other than those encountered in the blood and bone marrow have not been described.

The group at the National Institutes of Health [1046 1068] first reported severe leukopenia and a granulocytosis in rats when sulfaguanidine or sulfasuxadine was incorporated in the diet at a level of 1 per cent. Some of the animals showed reduction in hemoglobin and red blood cell concentrations These adverse hematological responses could be reversed by the administration of crystalline folacin More profound effects in rats have been produced by the use of the antagonist, 7-methyl folic acid [1069] Such animals exhibit slowing of growth, normocytic anemia, leukopenia and neutropenia. Studies of bone marrow revealed an increase in erythroid elements, particularly immature forms and a reduction in myeloid elements Necrosis of the lining of the oral cavity was also noted.

The most extensive and careful studies of folacin deficiency have been carried out in swine by Cartwright and his collaborators [1065, 1066] using an antagonist and sulfasuxadine in the purified diet whose protein quality and quantity were varied The hematologic picture which developed was characterized by leukopenia with a proportionally greater reduction of myeloid

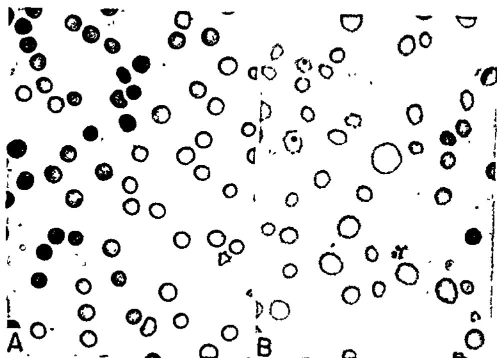


FIGURE 110 FOLIC ACID DEFICIENCY

Cartwright.)

cells than in monocytes and lymphoid elements, by a severe anemia which was macrocytic in type, by a slight thrombocytopenia, and by bone marrow hyperplasia with an increase in immature nucleated cells which resembled the megaloblasts encountered in Addisonian pernicious anemia (page 423). These hematological abnormalities responded to therapy with folacin. Purified liver extracts and vitamin B_{12} were only slightly effective in correcting the abnormal blood picture. Quantitatively the red blood count in the animals fell by 50 per cent or more. The volume of packed red cells also was reduced, the mean corpuscular volume rose, in one group from 54 to 84 cubic microns The mean corpuscular hemoglobin concentration increased slightly. No relationship was found between folacin deficiency and the metabolism of ascorbic acid or tyrosine. A decrease in free erythocyte protoporphyrin, an increase in plasma iron, and no particular change in plasma copper were described. Cartwright and his collaborators likened this anemia observed in swine to the group of non-Addisonian anemias discussed on pages 433 to 436.

Because of the adverse consequences of folacin deficiency on reproduction in the rat[1070] particularly in the presence of an antagonist,[1069] the effects on the young *in utero* have been most interesting[1071] In this study pregnant female rats were fed a synthetic diet containing 1 per cent succinylsulfathiazole and the antagonist x-methylpteroylglutamic plus folacin A deficient state was then produced in the fetus *in utero* at different stages of its development by removing the added folacin for varying intervals during gestation Periods such as the seventh to ninth day, the tenth to the twelfth day, et cetera, were studied

Multiple defects were found in virtually all areas· central nervous sys-

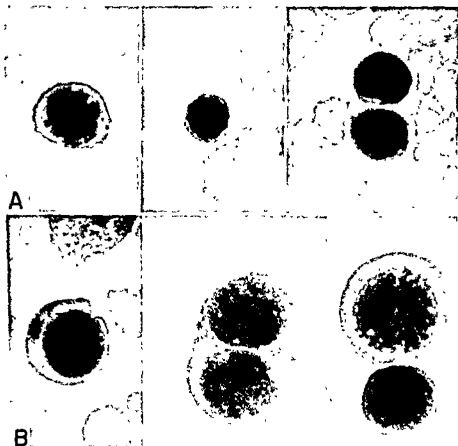


FIGURE 111. FOLIC ACID DEFICIENCY.

ls receiv-
Wright's

tem, eyes, lungs, heart and great vessels, urogenital system, diaphragm and body walls[1071] The type of anomaly and incidence varied with the duration of the deficiency and the time at which it was instituted.

In the urogenital area varied changes are found: renal and ureteral hypoplasia, hydronephrosis, hydroureter.[1072] The cardiovascular systems of 423 fetuses were studied; anomalies were found in 134.[1073] Since the cardiac mesoderm does not appear until the eighth day of gestation, changes were not encountered until the seventh to the ninth day at which time 28 per cent of the young *in utero* exhibited alterations. The incidence jumped to 57 per cent in the fetuses examined from the ninth to the eleventh day. This heightened incidence would appear to coincide with the development of the first aortic arch on the tenth day of gestation. No alterations in the cardiovascular system were encountered if the deficient state was produced in the mother after the tenth day. The following abnormalities were found: interventricular septal defects, persistent truncus arteriosus, double aortic arch, right aortic arch, absence of ductus arteriosus, absence of the entire arch, and various abnormal patterns in the origin of the subclavian arteries. The changes described are interesting and, of course, are similar to those produced in an analogous fashion by vitamin A deficiency (page 139).

The skeletal systems of these deficient fetal rats exhibit profound abnormalities[1074] Such include reduction in size of all bones, retardation of calcification sequences, and malformations reminiscent of those occurring naturally in the human. The following were found: fusion or lack of differentiation of phalanges, metacarpals, metatarsals and tarsals, disproportionate growth of one bone or group of structures to the skeleton, notching of the ribs, curvatures of the long bones (radius, ulna, tibia, fibula).

VITAMIN B_{12} (Cobalmin)

The story of the discovery of vitamin B_{12} began in 1926 when Minot and Murphy demonstrated that liver was efficacious in the treatment of pernicious anemia[1408] (page 430) Castle[1412] soon showed that the stomach played an important role in the pathogenesis of the disease. The effectiveness of liver was explained by the presence in it of an *erythrocyte maturation factor* (EMF) which was formed from two substances: an *extrinsic factor* derived from the diet and an *intrinsic factor* elaborated by the stomach. During the years, numerous attempts were made to isolate the *extrinsic* and *intrinsic factors* Progress was slow because it was not possible to produce a pernicious anemia-like syndrome in any species of animals, although experimental macrocytic anemia had been studied in monkeys[1041] Hence all investigations, particularly assays of active materials, had to be carried out on humans having the disease

In 1946, a new dietary factor, which was designated as factor X, was shown to be an essential nutrient for the rat.[1075] A particularly rich source of this factor is liver. When a microbiological test was developed to assay the amount of active material in the liver[1414] the story had almost been completed. For in the spring of 1948, a crystalline material was obtained from liver;[1415] this was soon given to patients with pernicious anemia. The blood picture returned to normal and the neurological changes were arrested.[1416 1417] Finally, cobalt was demonstrated to be an integral part of the new material which had been named vitamin B_{12}.[205] The structure of vitamin B_{12} proved to be so complex that its elucidation "has required seven years of patient and brilliant work, despite the use of mechanical and electronic calculating machines."[1076]

It is not possible at this time to specify the precise function or functions of vitamin B_{12} in the organism It would appear to be a fundamental nutrient for all cells, this may be due to its important role in nucleic acid metabolism in relation to thymidine synthesis, which in turn may be affected by its role in transmethylation[1052 1077]

The relation of vitamin B_{12} to methyl groups has been studied in rats Briefly, vitamin B_{12} is concerned with the *de novo* synthesis of labile methyl groups derived from formate, serine or glycine and the transfer of these to homocysteine to form methionine. It is not concerned with methyl transfer from methionine to choline or creatine. In the presence of vitamin B_{12} deficiency methionine synthesis is inhibited. Hence on a diet low in this amino acid protein synthesis is decreased. This will lead to deficits in other amino acids since they would be excreted in the urine.

In addition to the transmethylation activity just noted, vitamin B_{12} is important in the methylation of uracil to form thymine which in turn may be converted to thymidine which is utilized in the synthesis of desoxyribose nucleic acid. Its role in RNA formation is less clear though the formation of this type of nucleoprotein is decreased in vitamin B_{12}-deficient animals. Hence, this might affect protein synthesis also.

That vitamin B_{12} stimulates the conversion of glucose to ribose has been claimed [1078]

These metabolic effects of vitamin B_{12} are reflected in changes in the structure of tissues of animals deficient in this nutrient. Fatty livers, which develop in rats placed on low protein-low fat diets, are partially corrected by vitamin B_{12}.[1079, 1088] A similar protective effect is seen on the kidneys of choline-deficient rats where the incidence of typical lesions is lowered [1088] This evidently reflects the relation of vitamin B_{12} to choline metabolism On chemical analysis the livers of B_{12}-deficient rats contain decreased amounts of DNA and RNA.[1080] This is as might have been expected. Another effect which has been studied is the relation of vitamin B_{12} to CoA activity; the latter is reduced in the liver and kidneys of deficient animals.[1081]

Vitamin B_{12} deficiency has been studied in rats,[1080, 1082] guinea pigs,[1082] swine,[1085, 1086] mink [1087] and other species.

The most detailed studies of experimental vitamin B_{12} deficiency have been reported by Cartwright *et al* [1085, 1086] in swine. Of the thirty-nine animals which were studied growth was retarded in all. On the other hand prominent hematological alterations appeared in only a quarter. Such changes consisted of a normocytic anemia which was never particularly severe The bone marrow exhibited normoblastic hyperplasia. Neutropenia was present in the animals developing severe anemia and in some others in which the anemia was mild. Treatment with vitamin B_{12} elicited a prompt reticulocyte response.

The absence of any abnormal findings comparable to those seen in pernicious anemia led Cartwright and his co-workers to combine deficiencies of folic acid and vitamin B_{12} [1086] It will be recalled (page 273) that deficiency of the former leads to a macrocytic anemia and certain other hematologic abnormalities in swine. In twenty animals subjected to the double deficiency, macrocytic anemia, leukopenia due to neutropenia, and erythroid hyperplasia of the bone marrow were found. The cells in the bone marrow were large and resembled the megaloblasts of pernicious anemia more than those which had been seen in the previous study of folic acid deficiency alone. When folic acid was administered to the doubly deficient swine a prompt return of the blood to normal took place. The normal state persisted for several months, after which time a partial remission occurred.

When vitamin B_{12} was given alone, only a partial return of the hematologic values to normal was observed. When both folic acid and vitamin B_{12} were administered the response was prompt and lasting.

Similar studies of the dual deficiency of folic acid and vitamin B_{12} have been reported in guinea pigs [1062] This animal is, of course, eminently suitable because of the relation of vitamin C to folic acid (page 272). Megaloblastosis was produced by a deficiency of either nutrient alone or both together. Both macrocytes and microcytes were found in the peripheral blood, yet anemia was inconstant and did not become prominent unless vitamin C was withheld.

Congenital malformations have been described in the young born to vitamin B_{12}-deficient females.[1089, 1090] Such abnormalities appear to resemble, at least as far as they have been studied, the alterations associated with folic acid deficiency (page 275). Chemical studies indicate that the cytochrome oxidase content of the tissues of young born to vitamin B_{12}-deficient females is reduced [1090]

Part VII
Naturally Occurring Deficiency Disease

Part VII

NATURALLY OCCURRING DEFICIENCY DISEASE

INTRODUCTION

Thus far we have been concerned with the functional and anatomical alterations which are associated with deficiencies of single essential nutrients. Such deficiency disease states may be produced in virtually all laboratory animals with varying degrees of success So, too, many single deficiency syndromes have been produced in man. We must now take up the group of naturally occurring deficiency diseases, particularly those which have been observed in man. Here the transition from the cloistered laboratory to the world at large makes for all sorts of complications which confuse things no end Can one make any generalization concerning these naturally occurring disease syndromes?

Perhaps the most important point to realize is that most of these diseases result from a lack of *multiple* nutrients rather than deficit of a *single* essential. To be sure, certain diseases, such as scurvy in infants or iron deficiency anemia, may occur in a "pure" form, that is, uncomplicated by other deficiencies On the other hand deficiency states, such as beriberi, pellagra, the hypokalemic syndrome, rickets, et cetera, originate as a result of the presence of multiple deficiencies Some of these may be dietary in origin, others may be of the conditioned type, since so many derangements of structure and function, produced in any number of ways, may operate More and more attention is being given to the multiple nature concept of most deficiency disease syndromes, this should help to clarify our understanding of them and allow us to investigate them more intelligently.

In studying deficiency disease syndromes as they occur endemically or sporadically, one must take into account social, economic, geographic and other factors which affect peoples and their environment. Feeding and weaning customs, the types of foodstuffs ingested, as well as their variety and availability, all are important So, too, animate agents, water borne, insect borne, et cetera, may affect man or his livestock and often contribute to precipitating or worsening the clinically recognizable deficient state.

Many of the syndromes which will be discussed in this section have received much study and publicity, though we are somewhat vague as to their pathogenesis. As will be soon pointed out, our basic understanding of what beriberi was during the past and may be today is extremely fragmentary. So, too, our comprehension of the underlying disturbances in pellagra is practically nil. Before going on to discuss specific naturally occurring disease syndromes, some attention must be given to the most im-

portant deficiency disease, which until recently had been given only passing attention; we refer, of course, to starvation or semi-starvation For it is just as important to understand some of the effects of caloric undernutrition occurring naturally in the human as it was to be familiar with the nonspecific effects of inanition on the experimental animal, a subject which was discussed on page 11.

STARVATION

In this country the ravages brought about by famine and undernutrition are unfamiliar and seem very far away, though accounts of food shortages continue to find their way into the daily news, such as, for instance, the headline, FOOD NOW SCARCE IN INDIA'S NORTH, which appeared in *The New York Times*, April 21, 1957.

The studies of F G Benedict *et al.*[1099] and of Ancel Keys and his co-workers[12] on food restriction in experimental human subjects have done much to elucidate many of the physiological and structural alterations produced by starvation We shall draw freely on the latter contribution which is already a classic Another survey of human starvation when it is accompanied by edema has been contributed by McCance.[1100] The term starvation will be used as an all-inclusive one to refer to varying degrees of semi-starvation up to total food restriction. What are the effects of starvation?

The most outstanding change is loss of body weight in relation to a set of standards for age, height, et cetera. It must be emphasized that one's standards for "normal" may be somewhat different for individuals in North America as compared with the natives of Africa Weight losses up to 30 per cent of normal may be tolerated without fear since the normal can be regained following refeeding The lethal level of weight loss is usually set at about 40 per cent, although there are cases on record in which a loss of 50 per cent has been followed by recovery

With extreme loss of weight the external appearance takes on a rather specific character The skin becomes pale, thin, dry, inelastic and sometimes assumes a "grayish" color The face suggests ageing Gooseflesh-like areas of hyperkeratosis may be seen, particularly over the anterior surfaces of the thighs and upper arms A dirty, brownish pigmentation may appear almost anywhere. The hair is lusterless and dry The eyes appear dull and dead Blood vessels are diminished in the sclera.

As might be expected, marked loss of fat occurs in the subcutaneous areas which are visible externally. At autopsy,[1101] a similar decrease is found in the internal depots: about the intestine, mesentery, retroperitoneal, et cetera Wasting of the muscles is also seen, microscopic examination reveals atrophy of their fibers

The heart is usually decreased in size, though not many careful studies have been carried out This atrophy appears to coincide with the functional changes which may be observed· bradycardia, fall in blood pressure with weak pulse, decreased cardiac output, increased circulation time, and decrease in venous pressure. In German concentration and prison camps the

average heart weight of starved persons was 200 to 220 grams, a decrease of 25 to 30 per cent of normal. Studies on 492 individuals dying in the Warsaw Ghetto revealed heart weights ranging from 220 to 275 grams, or an average decrease of 20 per cent in size. Histologically, extreme degrees of "brown atrophy" may be encountered.

In severe starvation the brain and nerves appear to lose very little weight Histological alterations of a non-specific nature have been described, much of such changes may be complicated by postmortem autolysis. We cannot go into the alterations of personality and emotion which have been described. Individuals become apathetic, depressed, and introverted. They lose interest in sex and become preoccupied with food and with eating.

Gastrointestinal disturbances are common, these may be associated with the presence of animate disease agents in the host. Moreover, starving persons may eat anything that comes to them, hence irritating, nondigestible materials may be swallowed. Atrophy of the mucosa of the intestinal tract is common.

In other organs such as the liver, spleen, kidneys, mammary glands, adrenals and thyroid, atrophy is usually extreme. The adrenal cortex loses most of its stainable lipid. The skeletal system exhibits osteoporosis. Occasionally osteomalacia has been described. However, in order to have osteoid tissue, there must be osteoblastic activity. This is absent in starvation, hence the usual finding is osteoporosis.

Caloric restriction does not lead to any very dramatic morphologic changes other than atrophy. This was strikingly brought out in the prisoner of war and concentration camp studies carried out after the War in Europe. This is in contrast to the many syndromes, particularly of neurological interest, which we see among prisoners and internees in the Far East.

SALT DEFICIENCY AND THE LOW SODIUM SYNDROME

One of the pioneer studies of naturally occurring sodium depletion was made in 1915 by Holt *et al* [1104] in infants with diarrhea. In such subjects the excretion of water increased eight times while the outputs of sodium and potassium were nine and four times the normal, respectively. This was one of the important early studies of the loss of salt via the gastrointestinal tract

A second important contribution to the role of salt in naturally occurring disease was the study of Moss in 1923, who linked heat cramps with salt depletion as a result of excessive sweating [1095] When heavy muscular exercise is carried on at high temperatures, sweating is so profuse that much salt and water are lost. If only water is taken in the body sodium is further diluted and the syndrome of "heat cramps" is produced, this can be prevented if added salt is taken

A third fundamental advance in our understanding of the metabolism of sodium in disease came as a result of Robert Loeb's demonstration of the hyponatremia which is such a prominent biochemical defect in Addison's disease.[1102] During the past twenty years, a great deal more has been learned of the metabolism of sodium in disease, so that now one speaks glibly of "the low salt syndrome," "the hyponatremic syndrome," or "the low sodium syndrome." Much that is new has been derived from the ease with which serum sodium and potassium levels can be measured today by flame photometry, a far cry from the situation in the summer of 1935 when we drew blood from patients on Dr Loeb's service and then waited two days for serum sodium and potassium values to be determined

The low sodium syndrome may result from external loss, internal loss, internal segregation or deficient intake of the cation Another form of the syndrome is related to excessive retention of water and hence dilution of sodium if none accompanies the fluid Thus the state of the organism's water metabolism and the distribution of sodium in the various body compartments are of importance in assaying the pathogenesis of hyponatremia. So, too, possible alterations in other electrolytes, particularly potassium, may be significant. A classification of the causes of certain types of hyponatremia is presented in Table VIII.[1103, 1105]

What effects are associated with low serum sodium? Some of the prominent symptoms, which are derived from all of the major body systems are: weakness, apathy, semicoma, confusional states, nausea, anorexia, vomiting, cramps in the extremities, evidence of circulatory failure and uremia. Such

TABLE VIII

THE PATHOGENSIS OF THE LOW SODIUM SYNDROME

I *Decreased Intake*
  (a) Experimental restriction
  (b) Dilution of extracellular fluid by sodium-low fluids

II *Internal Loss*
  (a) Ascitic and edema fluid

III *Excessive Loss via the Kidneys*
  (a) Diuresis
  (b) Renal disease
  (c) Hormonal effects
    (aa) Adrenal.Addison's Disease

IV *Loss from the Gastrointestinal Tract*
  (a) Vomiting, suctional drainage
  (b) Diarrhea

V *Loss in Perspiration*

symptoms as these are not particularly specific and may be much overshadowed by the underlying disease which is causing the depletion of sodium

In the low salt syndrome the serum concentration of sodium is usually reduced by 15 to 20 mEq per liter. The serum bicarbonate and chloride levels are ordinarily similarly depressed. However, these may be modified by the primary cause of the hyponatremia; hence the chloride or bicarbonate may be disproportionately changed. Serum potassium values may be elevated or depressed, depending on the cause of the hyponatremia. In addition, rises in blood urea nitrogen or nonprotein nitrogen are found. The urine is usually decreased in amount.

THE HYPOKALEMIC SYNDROME

Reduction in the serum potassium concentration may occur in the human under a variety of circumstances. Experimental potassium deficiency in man, which has been produced by deliberate dietary restriction, is discussed on page 31. A number of situations arise in clinical medicine when the potassium concentrations of the serum may fall to low levels, sometimes with disastrous results A classification of the ways in which conditioned potassium deficiency states may come about is presented in Table IX. In

TABLE IX

THE PATHOGENESIS OF HYPOKALEMIA

I *Decreased Intake*
- (a) Experimental restriction
- (b) Starvation, followed by inadequate repletion
- (c) Dilution of extracellular fluid with potassium-free fluids

II *Excessive Loss via the Kidneys*
- (a) Diuresis
- (b) Acidosis (diabetes mellitus)
- (c) Renal disease
- (d) Hormonal effects
  - (1) Hypophysis (ACTH) Trauma
  - (2) Adrenal Cortisone cortisol, aldosterone

III *Loss from the Gastrointestinal Tract*
- (a) Vomiting, suctional drainage
- (b) Diarrhea, sprue syndrome

IV *Loss in Perspiration*

V *Excessive Transfer to Cells*
- (a) Glycogenesis (treatment of diabetes mellitus)
- (b) Familial periodic paralysis

any discussion of potassium one must remember that the amount of sodium which may be available to replace potassium is of importance. In addition, as Moore has emphasized, hypokalemia and tissue potassium depletion are not necessarily synonymous terms[96]

Ever since the experiments of Benedict forty years ago,[1099] it has been well recognized that starvation is accompanied by excessive losses of nitrogen, potassium, sulfur and phosphorus. The body, in effect, lives on and "consumes" its own protoplasm. With repletion, all of these elements must be supplied if the tissues are to be rebuilt This necessitates providing an abundant supply of potassium to the repletion regimen whether this is administered orally or intravenously.[85] Another situation is of importance to

the surgeon, to whom for many years the customary material for fluid replacement, particularly in dehydrated individuals, was normal sodium chloride, with or without glucose. The development of potassium deficiency in individuals so treated, particularly patients subjected to surgical procedures, has been clearly demonstrated, so that the addition of potassium to subcutaneous or intravenous solutions is thus indicated.[1106]

The main route of excretion of potassium is via the kidneys. A number of situations may increase the loss of potassium by this pathway and so produce hypokalemia and lowered intracellular potassium concentrations. Simple diuresis as a result of excessive oral or parenteral fluid administration is always to be thought of. So, too, acidosis, occurring as a result of derangement of acid-base balance or associated with renal disease, must be considered. That intrinsic kidney disease may lead to derangements in potassium excretion has been recognized for some time.[1107] We are concerned here with loss of potassium rather than retention of this ion. The various types of chronic nephritis: glomerulonephritis, vascular nephritis or pyelonephritis may lead to this change. The exact mechanism of loss in the urine is not clear. In such cases characteristic effects of potassium depletion on carbohydrate metabolism, protein metabolism, phosphorus excretion and sodium conservation may be demonstrated[1108]

Increased renal excretion of potassium is also seen in certain endocrinological disturbances, mainly those resulting from adrenal cortical hyperfunction. The increased urinary concentrations of potassium observed postoperatively is doubtless related to this, i.e., the "alarm reaction."[96] The administration of cortisone, cortisol, and desoxycorticosterone acetate produce similar effects, though to varying degrees Hypokalemia with alkalosis has been recognized as a prominent feature of the Cushing syndrome for some years.[1109] To this group has recently been added the syndrome of "primary aldosteronism" associated with adrenal cortical tumors.[1110]

Considerable concentrations of potassium are secreted into the stomach. Hence, loss of gastric secretions, due to vomiting from various causes or as a result of gastro-duodenal suctional drainage, may rapidly deplete the organism of potassium, particularly if none of this element is replaced So, too, loss of this cation from the lower intestinal tract as a result of diarrhea is of utmost[1111, 1112] importance. This is particularly brought out by Darrow's studies[1113] of diarrhea in infants. Naturally, other elements are lost as well, but improvement is far superior in those infants receiving potassium than in those to whom only saline is administered with or without glucose

One of the most significant advances in our understanding of the interrelationships of potassium and glucose metabolism has come from studies on individuals whose diabetic acidosis has been treated with insulin.[1114] It is clear today that certain of the failures of therapy which were unex-

plained at the autopsy table over a decade ago were the result of hypokalemia When glucose is being metabolized to glycogen and so enters the cell, potassium must necessarily accompany it Hence, if the diabetic organism has a depleted store of this cation as a result of vomiting or excess renal excretion associated with acidosis, not enough will be available to enter the cell, the disastrous effects of hypokalemia will ensue, particularly when fluids are given which expand plasma volume and increase renal output

The entrance of potassium into the tissues occurs in familial periodic paralysis; hence, serum potassium concentrations fall None is lost from the organism, either in the urine or stool [1115]

The most conspicuous *symptoms* of potassium deficiency are anorexia, nausea, muscular weakness, and mental depression Frequently present are mental confusion, shallow respirations and abdominal distention with paralytic ileus.[65] The *signs* of the hypokalemic state are mainly referred to the cardiovascular system, irregular pulse, fall in blood pressure, and



FIGURE 112 HYPOKALEMIC SYNDROME.

Kidney, human Sections from a patient [illegible]
death a few [illegible]
thelial cells
B (x 240)

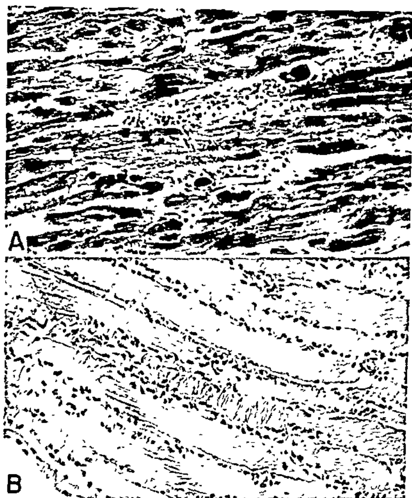


FIGURE 113 HYPOKALEMIC SYNDROME

Muscle, human *A* Section of cardiac muscle of patient shown in Figure 112 An area of necrosis infiltrated by inflammatory cells is present (x 200). *B* Section of striated muscle to show focal necrosis of fiber (x 200).

abnormalities in the electrocardiogram. These EKG changes are not specific for potassium deficiency but are of great importance in prognosis. They consist of prolonged QT interval, low T wave, sagged ST segment and depressed ST. With therapy such abnormalities revert to normal.[65] Individuals showing paralysis may have a loss of tendon reflexes.

The effects of hypokalemia on the human organism are difficult to demonstrate at autopsy. However, certain changes have been described in the heart and kidneys. This is reassuring in view of the alterations which appear in these organs in the experimental animal depleted of potassium. It will be

recalled that administration of *desoxycorticosterone acetate* leads to excessive excretion of potassium (page 28). Toxic effects of this hormone have been noted in man; these are evidenced by EKG changes[1116] Moreover, in at least one instance, fresh necroses have been found in the myocardium as a result of D C A administration.[1117] Thorn's group has commented on the presence of myocardial scars at autopsy in present-day cases of Addison's disease, a finding not noted before the introduction of hormonal therapy.[1118] McAllen[1121] has reported cardiac lesions in two individuals who had clinically exhibited severe and prolonged potassium depletion as a result of gastrointestinal disturbances Foci in the myocardium where fibers had lost their striation or were vacuolated together with cellular infiltrations and scars unrelated to blood vessels were described. The potassium content of the myocardium was assayed to be 15 mg per 100 gm, normal is reported as 300 mg. per cent A few other reports are also available[1119, 1120]

Renal changes, accompanied by derangement of kidney function, have been described in association with hypokalemic states,[1112] so that "*nephropathy of potassium depletion*" *appears to be a valid entity* Chronic diarrhea from a variety of causes would seem to be of importance in the pathogenesis of the potassium depletion. Evidences of renal functional impairment are reduction in clearances of insulin, endogenous creatinine, and para-aminohippuric acid Proteinuria and cylinduria are inconstant findings. Blood non-protein nitrogen values are not particularly elevated

On biopsy or at autopsy alterations similar to those observed in rats may be encountered in the tubular epithelial cells Biopsy studies have shown a restoration of the cells to normal following appropriate therapy with potassium The potassium depletion syndrome appears to furnish a good example of renal disease which is amenable to therapy

TETANY

A syndrome of convulsive seizures, associated with fibrillary twitchings, and spasms of the muscles of the extremities and larynx, has been recognized in infants, particularly those exhibiting rickets, for at least two centuries. A similar condition in adults became prominent during the early days of surgical ablation of the thyroid gland for toxic goiter. The importance of the parathyroid glands in the maintenance of normal serum calcium concentrations and the role of these ions in affecting the irritability of the neuromuscular apparatus was reported by MacCallum and Vogtelin in 1909[151] Hypocalcemia was shown to be a cause of infantile hyperirritability by Howland and Marriot in 1918[1146]

The increased irritability of the neuromuscular apparatus, which is evinced by fibrillary muscular twitchings and spasms, is called tetany. From what is known of the role of inorganic ions in neuromuscular activity one might expect hyperirritability when imbalance in the concentrations of the cations calcium, magnesium, potassium and sodium, are present. All of these ions are concerned with maintaining what must be critical levels for the support of activities of nerves and muscles. In Table X are listed

TABLE X

THE PATHOGENESIS OF TETANY

I *Calcium Deficiency*
- (a) Deficient intake
- (b) Disturbances in absorption including vitamin D deficiency and diarrhea
- (c) Excessive excretion by kidneys
- (d) Formation of complexes
- (e) Parathyroid Disease
  - (aa) Maldevelopment
  - (bb) Removal
  - (cc) Pseudohypoparathyroidism

II *Alkalosis*
- (a) Hyperventilation
- (b) Vomiting, pyloric obstruction

III *Magnesium Deficiency*

IV *Potassium Deficiency*

the abnormal states which may give rise to the tetany syndrome Hypocalcemia was the first derangement to be coupled with the convulsive fibrillary twitchings and spasm which characterize tetany. This may be brought about as a result of dietary lack, poor absorption, parathyroid disease or renal disturbances. When calcium-deficient diets are fed to laboratory ani-

mals, spontaneous tetany is ordinarily not encountered. However, hyperirritability may be demonstrated by stimulating the animal by electric shock or loud noises. Fatal tetany may be induced in this way.

Intestinal dysfunction, such as is seen in the malabsorption state (page 437) which characterizes sprue, celiac disease and steatorrhea, may lead to hypocalcemia and tetany. This diarrhea compounds things by causing excess loss of vitamin D, which further effects a reduction in calcium absorption The formation of insoluble calcium compounds such as oxalates and phytates may promote calcium deprivation.

Certain renal diseases are characterized by hypercalcuria Two of these may be due to inherent tubular defects: one dealing with the reabsorption of calcium, the other with acid-base function. These are the syndromes of idiopathic hypercalcuria [152] and renal tubular acidosis.[1314] The other situation is seen when glomerulotubular disease is present, i.e., glomerulonephritis, vascular nephritis or pyelonephritis Here the primary derangement appears to be the phosphate retention which supposedly leads to a reciprocal reduction in serum calcium concentrations. It must be pointed out, however, that whenever serum phosphorus concentrations rise or fall an opposite change in calcium is not inevitable So, too, alteration in phosphorus concentration does not always follow primary changes in calcium values

The intravenous or parenteral administration of simple or complex compounds which may combine with calcium will lead to hypocalcemia. Hence, vascular or intraperitoneal injections of phosphate, oxidate, citrate or chelating agents such as EDTA will precipitate tetany in experimental animals.

Finally, parathyroid hypofunction is an important factor in leading to reduced concentrations of ionized serum calcium. This may be brought about as a result of nondevelopment of the parathyroid glands [1149] or their operative removal,[151] either purposely or inadvertently. The syndrome of pseudohypoparathyroidism is charcterized by low serum calcium levels which fail to rise to normal following the administration of parathyroid hormone [1150] The basis for this phenomenon is not known.

It will be noted that situations other than hypocalcemia, *viz*, alkalosis and magnesium or potassium deficiencies may lead to tetany. It has long been recognized that gastric or pyloric obstructions with vomiting and chloride depletion, lead to increase in serum bicarbonate concentrations and tetany.[1151] So, too, the blowing off of carbon dioxide by overbreathing may lead to alkalosis and tetany.[1152] The exact cause of the tetany is not entirely settled; it has been assumed that increased hydrogen ion concentration of the plasma leads to depressed ionization of calcium The entire problem is a most complex one [1266] which cannot be discussed further here

Two other forms of tetany have been recognized The classic observations of McCollum *et al.* on animals deprived of magnesium together with studies of the naturally occurring disease in calves have been mentioned on page 35. Cases which have been designated as magnesium tetany have been reported from time to time in the human For instance, suspected magnesium deficiency has been reported in a child [1153] Convulsions were first observed at seven months of age. These gradually decreased, to be followed by dizziness at age three years and a tremor which began a little later and increased in severity Tetany was noted, serum magnesium values were low Magnesium therapy abolished the dizziness and tremor, and both returned when treatment was discontinued. Magnesium deficiency has been described in an adult,[1155] in whom confusion, stupor, tremors and othetoid movements were noted after severe diarrhea. Other electrolyte disturbances, i e, calcium and potassium may have been implicated It is of interest that neuromuscular abnormalities other than weakness have not been found in experimental subjects who have been subjected to dietary magnesium restriction [1433]

Finally, the role of potassium must be mentioned. A number of years ago we observed peculiar spontaneous tetanic contractures of the muscles of the extremities of animals which had been placed on potassium-low diets and made to swim for prolonged periods [74] When removed from their swimming tank and placed on the floor, "they jumped into the air to a height of about a foot These spasmodic contractures were repeated 10 to 12 times after which the animals lay exhausted" Unfortunately no blood studies were carried out on these rats Recently potassium deficiency tetany has been discussed by Fourman [1154] who studied the experimental depletion of human subjects and also noted the syndrome in naturally occurring hypokalemic states. Increased irritability of nerve trunks was noted. Small reductions in serum calcium and mild alkalosis were also noted but were not deemed sufficient to lead to hyperirritability In fact, hypokalemia would have been expected to protect against tetany Here again nothing is known of the potassium content of the intracellular fluid or that immediately adjacent to the cells

IRON DEFICIENCY ANEMIA

Experimental iron deficiency leads to striking changes in the red blood cell count. It is not surprising, therefore, that iron deficiency anemia may be encountered in both children and adults. The causes of the deficiency are many In children dietary intake is most often at fault, particularly in bottle fed babies since cow's milk contains so little iron. This form of nutritional anemia is the most common type of anemia after early infancy.[1122] In adults inadequate intake may also play a role though increased requirements, as in pregnancy[296] and, more particularly, excessive loss, are of great importance.[1123]

In children and adults, iron deficiency anemia is characterized by microcytosis and hypochromia Several clinical types should be mentioned[1123] Chlorosis is an example of anemia which results from inadequate intake of iron, this syndrome, which used to occur primarily in young women, is now uncommon As has already been mentioned (page 64), achlorhydria may affect the absorption of iron, so that many cases of hypochromic anemia, especially in women, commonly show a decrease or absence of hydrochloric acid in the gastric contents. So, too, when the passage of ingested materials through the intestinal tract is rapid, iron may be poorly absorbed, hypochromic anemia is often encountered in cases of chronic diarrhea. During pregnancy, an increased requirement for iron develops in order to supply the fetus, at this time, therefore, hypochromic anemia may be observed[296] Chronic blood loss from any site, sometimes coupled with insufficient iron intake, is the most important factor in the production of hypochromic anemia Hemorrhage from the gastrointestinal tract as a result of a variety of lesions including hookworm infestation and loss during menstruation play extremely important roles in this connection In all of the clinical syndromes briefly eluded to, treatment with iron provokes a prompt reticulocyte response and a return of the blood picture to normal. Of course, in order to have the red blood and hemoglobin concentration remain at the normal limit, the underlying causative mechanism must be eradicated.

Since virtually no uncomplicated cases of clinical hypochromic anemia come to autopsy, the pathological changes in the tissues, if any, are not clearly understood During life, the bone marrow exhibits normoblastic hyperplasia[1123] In view of the scant pathological changes observed in experimental animals as a result of iron deficiency, certain peculiar changes which are seen in iron-deficient anemias in the human deserve mention. Sore tongue and sore mouth similar to the changes encountered in un-

complicated nicotinic acid, riboflavin and pyridoxine deficiencies (pages 220, 218 and 248) have been described, such lesions are said to respond to therapy with iron.[1124] In addition, extreme dysphagia may be present; this is called the Plummer-Vinson Syndrome.[1123] Another interesting finding is the development of koilonychia or longitudinal ridging and flattening of the fingernails, which may even change from the normal convex to a concave form.

ENZOOTIC COBALT AND COPPER DEFICIENCIES

During the past twenty-five years much effort has been directed toward clarifying certain diseases of world-wide distribution in cattle and sheep, which appear to be related to specific mineral deficiencies of the pasturages which these animals were utilizing The deficiencies of copper and cobalt may be taken up together, since in early studies confusion often arose between the two In the following discussion we shall draw freely on the reviews of this subject by Marston[1125] and Underhill[1126]

The story of the recognition of cobalt deficiency in farm animals is one of the triumphs of veterinary medicine The disease syndrome is characterized by progressing and profound wasting, accompanied by a severe anemia and by a lack of appetite The sheep exhibit extreme emaciation with pale skin and mucous membranes Examination of the blood reveals a macrocytic anemia This syndrome was first studied by Filmer[1127] in Western Australia Here it was called "enzootic marasmus" Although iron salts and liver were at first shown to be effective in curing the disease, subsequent work revealed that impurities in the iron-containing limonite salts were the active agents[1128] Of these cobalt was specific[1129] This element is found in insufficient quantities in the pasturages upon which the affected animals feed

While these investigations were being carried out in Western Australia, similar studies were being pushed in the southern part of that continent In 1935 Marston[1130] reported a wasting disease in sheep in which there was an anemia The administration of cobalt led to a marked gain in weight, together with an elevation of hemoglobin concentration and red blood cell count.[1131] This malady, "coast disease," as it was called, was accompanied in some instances by ataxia

Utilizing the Marchi method, which is an unreliable one, degeneration and demyelinization of certain tracts in the spinal cord were demonstrated[1132] In affected animals histological examination of the tissues revealed increased deposits of hemosiderin in the liver, spleen, pancreas, and kidney, on chemical analysis iron was found in large quantities in these organs[1133] "Coast disease" was then shown to be caused by a deficiency of both cobalt and copper[1134] The neurological lesions appear to be related to those encountered in uncomplicated copper deficiencies of sheep, which will be described below.

A disease similar to "enzootic marasmus" has been reported in Florida[1135] The muscles of affected calves are pale, much body fat is lost. "De-

generative" changes are found in the heart; the spleen in said to be shrunken, the liver is fatty.

The initial studies of sheep on cobalt-deficient pasturages indicated that in order to be effective, the missing element must be administered frequently. Moreover, it was found that cobalt must be ingested, parenteral administration was valueless.[1136] The exciting demonstration that cobalt is a part of the vitamin B_{12} molecule[205] has led to a clearer understanding of the pathogenesis of these enzootic diseases in sheep and cattle. Large amounts of parenteral vitamin B_{12} were found to be effective. The entire story has been clarified by the demonstration that exceedingly large quantities of vitamin B_{12} are synthesized in the rumens of these animals[1137] Thus cobalt becomes a limiting factor in the synthesis of this vitamin. It would now appear that, although the needs of sheep and cattle for vitamin B_{12} are exceedingly large, in the presence of a normal intake of cobalt their flora can supply the necessary quantity of the vitamin.

The first evidence for the relation of copper deficiency to the health of grazing animals came from the Netherlands.[1138] Since then, copper deficiency disease of cattle has been described from various parts of the world· Florida, Australia, England, Scotland, Ireland, Norway, Sweden, and New Zealand.[1125, 1126] The syndrome of copper deficiency in cattle is characterized by changes in the coat; the hair lacks pigment and becomes harsh and disheveled.[1139] The bones may spontaneously fracture There is anemia. Reproductive capacity is impaired, as is milk production. Serum copper concentrations are reduced. At autopsy extensive hemosiderosis of the spleen, liver, and kidney is found. Lesions have been described in the kidney and myocardium. In the former tissue, degeneration of glomeruli is said to be prominent. The tubular epithelium shows cloudy swelling while the lumens are filled with detritus. From the available description it is difficult to come to a decision as to the exact nature or pathogenesis of these renal changes. The hearts of affected cows show extreme fibrosis, which has been interpreted to result from atrophy of the muscle fibers due to longstanding anoxemia (starvation atrophy). Neurological lesions are not prominent Copper administration clears up these various manifestations of the deficient state.

In sheep, studies of spontaneous or endemic copper deficiency have been reported from Australia[1140] and England.[1141] The disease in this species is called "enzootic ataxia" or "swayback." Although the importance of copper in the pathogenesis of this malady may be considered equivocal by some until specific experimental studies are carried out with purified diets, it would seem that swayback must be due to a deficiency, a conditioned deficiency, perhaps, of copper. In Australia the syndrome occurs only in those pasturages whose copper content is low, although this important point is

not confirmed by English investigators. Determinations of the blood copper content of pregnant ewes has generally revealed decreased concentrations of this element.[1142] As was first shown by Bennetts and Chapman [1140] in Australia, and subsequently confirmed by Dunlop *et al* [1141] in England, the incidence of swayback can be greatly reduced, or even eliminated, by the administration of copper salts [1142] Further studies have shown that other trace elements, such as iron and cobalt, are ineffective, and that treatment with copper raises the concentration of this element in the blood of pregnant ewes [1143] The most marked changes have been found in the newborn lamb. The clinical signs consist of spastic paralysis, especially of the hind limbs, severe incoordination, and, in some instances, blindness The maladay is seen only in newborn or very young lambs, in some flocks the incidence has been as high as 90 per cent of all the animals born The pathological changes in lambs exhibiting manifestations of swayback were reported by Innes in 1934,[1144] before the relationship of copper deficiency to the syndrome had been fully elucidated Innes has since extended his studies to the nervous tissues of a large group of lambs affected with the disease and has carefully described the extraordinary changes [1145]

The external appearance of the brain is striking since this structure is smaller than that of a normal newborn lamb. In addition, there is depression

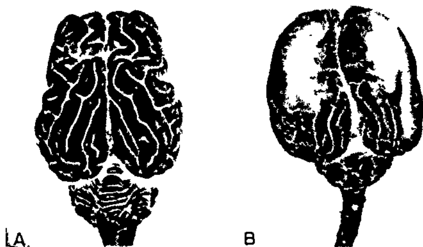


FIGURE 114 ENZOOTIC COPPER DEFICIENCY

Brain, sheep. Gross appearance of brain, *A* from normal newborn lamb, and *B* from an animal dying three days after birth with signs of "swayback" Note collapse of cerebral hemispheres and shallow convolutions (Courtesy of Dr J R M Innes and *The Journal of Comparative Pathology and Therapeutics*)

generative" changes are found in the heart; the spleen in said to be shrunken; the liver is fatty.

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In sheep, studies of spontaneous or endemic copper deficiency have been reported from Australia[1140] and England.[1141] The disease in this species is called "enzootic ataxia" or "swayback." Although the importance of copper in the pathogenesis of this malady may be considered equivocal by some until specific experimental studies are carried out with purified diets, it would seem that swayback must be due to a deficiency, a conditioned deficiency, perhaps, of copper. In Australia the syndrome occurs only in those pasturages whose copper content is low, although this important point is

descending tracts has been demonstrated in the spinal cord. In those lambs in which clinical evidence for the presence of the disease is slight, the neurons usually show no change, while in those exhibiting more severe symptoms, degenerated cells are found, a constant site of damage is the red nuclei. No inflammatory reaction is evident in the tissues about blood vessels, save the presence of mononuclear phagocytes filled with lipoid. Innes has called attention to the similarity of the pathological manifestations of this condition in lambs to Schilder's Disease in man. His photographs which he has so kindly allowed us to reproduce confirm this similarity.

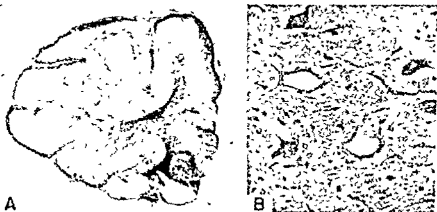


FIGURE 116 ENZOOTIC COPPER DEFICIENCY

Brain, sheep. *A* Section from occipital pole to show loss of myelin with cavity formation at several points. Weigert-Pal (x 4). *B* Higher power of neurons in red nucleus, two of whose cells show chromatolysis. Nissl-Orange G method (x 400). (Courtesy of Dr J R M Innes and *The Journal of Comparative Pathology and Therapeutics*.)



FIGURE 115 ENZOOTIC COPPER DEFICIENCY.

Brain, sheep. Coronal sections A. through brain of normal two-day-old lamb, and B animal dying five days after birth with characteristic signs of "swayback" Note extreme destruction of white matter with cavity formation and wasting of the corpus callosum The gray matter is relatively well preserved The ventricles of the affected brain appear to be dilated (Courtesy of Dr. J. R. M. Innes and *The Journal of Comparative Pathology and Therapeutics*)

of the cerebral hemispheres due to the apparent loss of substance beneath. On section, the gross lesions vary from small foci of porencephaly in the white matter of the central hemispheres to areas in which the central white matter is restricted to a "grossly degenerated centrum ovale, to a wasted *corpus collosum* and *septum pellucidum* and to the internal capsule." In contrast, the cerebral gray matter is relatively well-preserved and forms a thin shell around the degenerated white matter of the cavity.

Microscopically, symmetric diffuse demyelinization is found in the white matter of the cerebral hemisphere. In areas of demyelinization the axis cylinders disappear, moderate glial proliferation is found in and about such areas. Destruction of myelin has not been encountered in the midbrain, cerebellum or brain stem, but, as might be expected, degeneration of the

ENDEMIC GOITER

Swelling of the neck, or goiter, has been recognized from earliest times. Its geographic distribution and its relation to the iodine content of the soil and drinking water have been commented upon at length during the last century. Prophylaxis with iodine compounds, changes in dietary habits, and the multitudinous influences that go to promote "civilization," all appear to have changed the pattern of distribution of goiter in the world today. Be this as it may, the Study-Group on Endemic Goiter, which was convened by the World Health Organization in London in 1952 [1543] pointed out that "recent surveys have shown that the incidence of endemic goiter is far more extensive than was formerly realized" and further that in certain areas "the disease constitutes a social, economic, and health problem of great magnitude." Hence, it is of the utmost importance to discuss endemic goiter and to take stock of the present state of our ignorance concerning this problem. We should like to answer as best we can the following questions: (1) What is the pathology of endemic goiter, and (2) What is the pathogenesis of this change?

In recent years pathologists appear to have directed their attention to tumors to the exclusion of other changes in the thyroid gland. With certain exceptions very little material has been published concerning the status of normal or diseased non-neoplastic thyroid tissue. Hence, it is necessary to go back several decades and review Marine's,[1156] McCarrison's,[1157] and Aschoff's [1158] contributions to the goiter problem in order that certain fundamental principles may be understood.

Before doing this, the striking geographic distribution of goiter needs to be mentioned. It is now well-recognized that goiter is associated with those mountainous reigons whose soils were deposited during the last great glacial period. Such areas include the Alps, Pyrenees, Carpathians, Himalayas, Andes and Rockies. Other locales, such as the Thames Valley in England, certain regions of Africa, New Zealand and Australia, and the Great Lakes basin, have also been associated with a high incidence of goiter. We cannot hope to designate specifically those areas in various countries where goiter has been found to be most prevalent. The following list with references may be helpful. United States,[1159, 1160] Mexico,[1161] El Salvador,[1162] Venezuela,[1163] Argentina,[1164] Switzerland,[1165] Hungary,[1166] Yugoslavia,[1167] Nigeria,[1168] Sierra Leone,[1169] Union of South Africa,[1170] India,[1171] Australia,[1173] and New Zealand [1172, 1173]

We can now turn to the fundamental morphological alterations which have been described in the thyroid glands of individuals living in areas

parently most frequently seen in specimens removed at operation or at autopsy in children dying of natural causes in goiter areas.

If tissue from a diffuse goiter removed from an adult is studied, the only difference which may be found is a reduction in the size of the follicles. This goiter Aschoff called the *diffuse microfollicular colloid* type. The epithelium remains hyperplastic in appearance.

Finally, the variable picture of classical colloid goiter, with all degrees of localized epithelial proliferation, with or without adenomatous nodules, cysts, hemorrhage, scarring, and calcification, is seen in the adult and becomes more marked in the older age groups. Aschoff looked upon these stages in the evolution of endemic goiter as accentuations of the normal physiological responses of the gland: hyperactivity in the newborn, regression during infancy, enlargement during pre-puberty and puberty, regression in post-puberty, normal gland of the adult and atrophy in the decline of life.

As already noted, it is unfortunate that during the 30 or more years since the publications of Marine,[1156] Aschoff[1158] and McCarrison[1157] relatively little attention has been given to the morphologic aspects of endemic goiter. Do the alterations which have just been mentioned in the newborn, adolescent, and adult glands continue to be found in areas where goiter is endemic? Numerous questions might be asked concerning the

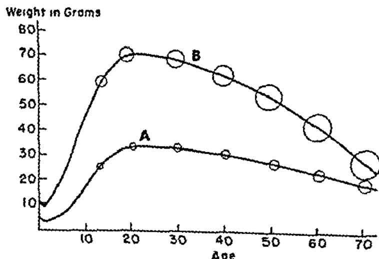


FIGURE 118 ENDEMIC GOITER

Graphs modified from Aschoff[1158] to show differences in weights of thyroid glands from a nongoiterous region, *A* and goiterous areas *B*. In addition, the size of nodules is shown by the diameter of the circles along each of the curves.

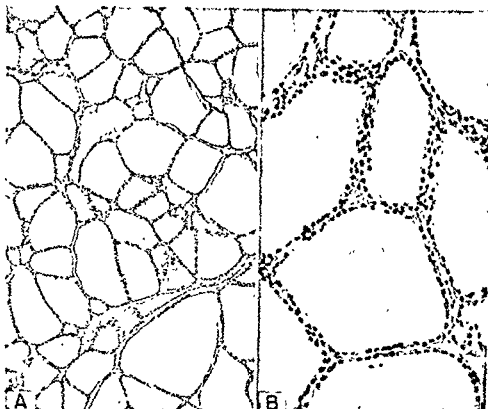


FIGURE 117 NORMAL THYROID GLAND, HUMAN.

A and *B*, (x 60) and (x 235), respectively, to show size and shape of follicles and appearance of lining epithelial cells

where goiter is prevalent. The deviations from the normal which may be observed in the thyroid gland at autopsy on newborns from goiter districts are definite though not great. Ordinarily the gland weighs 5 to 6 grams more than normal; occasionally it may reach a total weight of 100 grams. Microscopic examination reveals diffuse hyperplasia; that is the cells are increased in number and size, while the follicles contain a scanty amount of colloid. This anatomical picture is that of *diffusely hyperplastic goiter of the newborn.*

During the period of adolescence to puberty the gland may continue to enlarge visibly. Moreover, it shows characteristic microscopic changes The main alteration is an increase in size of the follicles; these may be visible to the naked-eye and hence may be termed macrofollicles. The epithelium lining such follicles is flattened. Only occasionally are foci of papillamatous hyperplastic cells found growing into the colloid space. This type of goiter, which Aschoff called *diffuse macrofollicular colloid goiter,* is ap-

deficiency. This observation is in keeping with present-day knowledge of thyroid physiology and the results of experimental iodine deficiency studies which have been discussed on page 78 From this morphologic state Marine postulated two courses which were open to the thyroid gland The hyperplasia might continue, ultimately to be followed by "exhaustion atrophy" Exactly what the picture of "exhaustion atrophy" is has never been entirely clear Nor has anything approaching this been observed in experimental animals which have been placed on iodine-deficient rations The second course might be followed if iodine became available to the gland, then the follicles fill up with colloid material This state, or colloid goiter, Marine considered to be the closest one to normal which the gland could reach, he regarded it as a quiescent stage The gland might remain in this phase unless iodine deficiency recurred, or some other stimulus to hyperplasia returned. The same chain of events might then be repeated Such is the "Marine cycle", normal gland→hyperplasia→colloid goiter This has been a most stimulating hypothesis, although these sequences have not been demonstrated in the experimental animal Nevertheless the observations and this hypothesis of Marine have led to a number of experimental, epidemiological and prophylactic investigations which have been of utmost importance

The experimental production of hyperplastic goiter in animals has been mentioned elsewhere (page 78) Since, as is clear today, the thyroid gland is under the control of the hypophysis, anything which will lower the plasma concentration of active principle of the thyroid will lead to excessive TSH production and so result in thyroid cell hyperplasia Of these factors iodine lack is certainly of importance Others such as excessive intake of calcium have been mentioned [253] A possible role of fluoride has recently been proposed [1170] The significance of anti-thyroid goiterogens will be discussed below

Over 100 years ago, in 1820, Coindet suggested that goiter in the human might be related to iodine deficiency In 1850 Chatin found a low iodine content in the soil and water of areas where goiter was prevalent This study was disregarded until Marine's observations redirected attention to the possible role of iodine deficiency in the production of goiter. A number of investigations have shown the relationship of low content of iodine in water and soil to goiter in the United States and other areas These studies have been summarized by McClendon,[1159] who appears to conclude that the cause and effect have been conclusively shown Most recent surveys bear this out (page 307)

Finally, the studies of Marine led to the introduction of iodine as a prophylactic agent in the prevention of goiter Iodine will not, of course, decrease the size of a large colloid goiter once it has formed The prevalence

morphologic appearance, iodine content, et cetera, of present-day goiters. Should not studies, such as those performed by Aschoff and others, be repeated, particularly before the salt everywhere is iodinated? A striking example of the neglect of morphologic studies is found in the report of the Mendoza expedition [1164] Not a single piece of tissue from any patient who was clinically and biochemically studied was examined under the microscope.

Clinically, goiter manifests itself as a swelling of the neck.[1174] This may become extreme, the gland reaching many pounds in weight. Aside from cosmetic effects, which many individuals do not appear to mind, the goiters lead to little discomfort. There is one disease which is closely associated with endemic goiter, this is cretinism The number of cretins in such an area as Switzerland, where goiters have been so prevalent, is high. This form of hypothyroidism in children appears to be associated with goiter and well it might be if iodine deficiency is the cause of thyroid enlargement. The prophylaxis of goiter with iodine appears to have reduced the incidence of endemic cretinism

How are we to explain the pathogenesis of the changes which have been described? Numerous pieces of evidence point to iodine deficiency as a prominent cause of goiter, yet as a single etiological factor this cannot answer all of the complex questions which arise.

Much of today's understanding and teaching of the pathogenesis of endemic goiter stems from the observations of David Marine and his co-workers.[255, 1175 1176, 1177, 1178] Whether the goiter hypothesis of Marine will stand up remains to be seen. However, he has done much to stimulate study and to clarify many facets of the goiter problem

It will be recalled (page 77) that Halsted [254] noted hyperplastic goiter in pups born to dogs from which he had removed the thyroid gland: Marine and Lenhart [255] demonstrated that such goiters in the pups could be prevented by administering iodine to the bitch during the course of her pregnancy. Marine and his co-workers then went on to show a close relationship of iodine concentration in thyroid tissue to the anatomical structure of the gland in fish, swine, dogs and man. One fact was clearly demonstrated. when the iodine content of the thyroid gland falls below a critical level of approximately 1 mg. per gram of dried gland, hyperplasia is to be expected. Such an hyperplastic change consists of enlargement and proliferation of the epithelial cells lining the follicles and a decrease in stainable colloid. Moreover, the gland becomes more vascular. This change is not ordinarily associated with evidences of clinical hyperfunction. Histological study of large numbers of thyroid glands from dogs, swine, cattle and man convinced Marine that the thyroid responded to iodine lack by hyperplasia. Such a change was felt to be the primary response to iodine

of goiter has statistically been reduced in areas where iodine has been added to the dietaries via salt, water, et cetera. This evidence would appear to be important in relating iodine deficiency to the pathogenesis of goiter.

The introduction of radioactive iodine (I^{131}) as a tool with which to investigate the physiology of the thyroid gland has also contributed to our understanding of endemic goiter. A study which was carried out in Mendoza province of Argentina on goiterous individuals who were subsisting on a diet inadequate in iodine has shown that the thyroid glands of such an iodine-deficient group cleared iodine more rapidly from the blood than did the normal. Moreover, those who had a high uptake of iodine by the thyroid gland exhibited a low urinary excretion of this element. These results led the group to state that "any remaining doubt that iodide deficiency can be a cause of endemic goiter has been erased by the present studies This is not to say that iodide deficiency is the cause of all endemic goiter, for it is quite possible that unrecognized goiterogenic substances may cause goiter by creating a block of utilization of iodide for hormone production."

In view of present-day knowledge of goiterogenic agents and because of certain discrepancies in the iodine-goiter relationship the above statement would appear to be a most sensible one The whole question of the production of endemic goiter in man by mechanisms other than iodine deficiency has taken on new significance since the discovery of antithyroid agents such as cyanate, thiourea and aminobenzene compounds. At least one goiterogen, 1-s-vinyl-2 thiooxazolidone has been isolated from naturally occurring foodstuffs belonging to the cabbage family and has been shown to affect uptake of I^{131} by the human thyroid gland [1179] Recent studies [1180] appear to implicate a thyroid blocking agent in the production of goiter in Tasmania Future investigations here and elsewhere are certainly indicated

Another group of goiters have recently received attention and deserve mention These are instances of goiter associated with sporadic cretinism

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FIGURE 119 GOITER, HUMAN

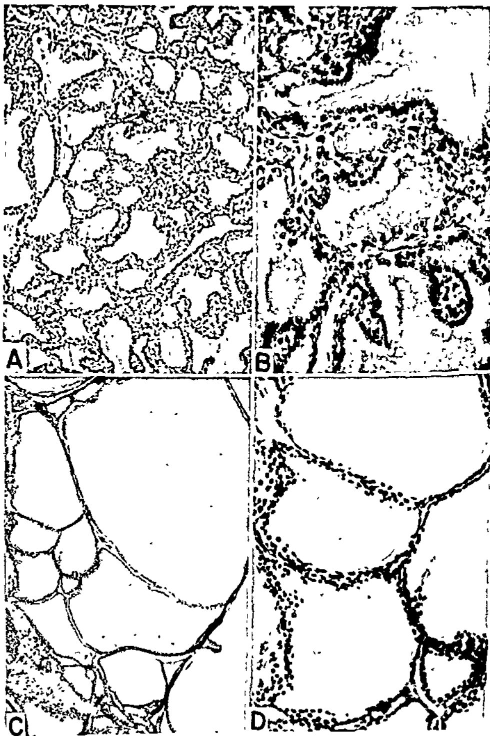
A and *B*, low (x 60) and higher magnification (x 235), from hyperplastic goiter removed from a thirty-seven year old woman because of choking sensation, swelling of the neck, nervousness and bulging of the eyes BMR, +60 Treatment, with KI reduced BMR to +13 at which time gland was subtotally removed Note infolding of epithelium which is columnar. Colloid is scanty. This is, of course, an example of exophthalmic goiter The hyperplasia would be indistinguishable from that seen if hyperfunction were not present Compare with normal, Figure 117. *C* and *D* Low (x 60) and high (x 235) magn [illegible]

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[illegible] normal, Figure 117


A
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PROTEIN DEPLETION SYNDROMES

INTRODUCTION

In Part III we briefly alluded to the many roles of protein in the organism Now we should like to discuss certain naturally occurring disease syndromes which may be associated with a decrease or even absence of one or more of the proteins which are so necessary for the integrity of the organism

The indispensability of certain amino acids for the synthesis of protein is well-recognized Hence dietary intake of these essentials is of the utmost importance Just as vital is the proper absorption of amino acids if protein is to be synthesized Hence, various gastrointestinal disturbances may lead to protein depletion. Protein may be lost by bleeding and by excessive excretion in the urine

We cannot go into the many genetic protein disturbances which center on the gene-enzyme hypothesis. All such situations can, of course, be regarded as examples of specific endogenous protein deficiency states in the sense that lack of an enzyme is responsible for a disturbance in the metabolism of an element, of an amino acid or other nitrogenous material, or of a lipid or carbohydrate.

We shall be concerned here with certain protein disequilibrium states. Some are clearcut enough to warrant being called diseases or syndromes. Such include pellagra, blacktongue and kwashiorkor Others are more nebulous or actually comprise specific parts of certain disease syndromes such as the depletion states which accompany the sprue syndrome or the nephrotic state One is closely associated with general undernutrition, i.e., starvation, yet is historically of great importance and has done much to focus attention on the problems of protein malnutrition. This entity is, of course, nutritional edema or hunger edema

HUNGER EDEMA

War, pestilence, and famine have always gone hand in hand. The ravages of the former two have always overshadowed the latter. As a matter of fact, de Castro[1098] points out in his *Geography of Hunger* that famine has been a routine matter in many areas for centuries He cites the fact that, during a 2000 year period, 1829 famines were recorded in China—an average of almost a famine a year The subject of hunger edema is intimately a part of caloric undernutrition and its pathogenesis is far more complex than simple hypoproteinemia. Yet lowering of plasma protein concentrations is one important factor and justifies inclusion at this point.

McCance[1100] has presented an admirable account of nutritional or

which have been studied by Stanbury[1434] and others. Such goiter may result from blocks in the metabolism of iodine in the thyroid gland itself. If one reviews the schematic representation of the pathways of iodine in the thyroid gland (Figure 38, page 75), he may guess that defects in the enzymes controlling the various reactions might occur. So they do Already several such defects have been encountered such as inability to oxidize iodide to iodine or to couple iodotyrosines to iodothyronine. A third defect deals with loss of mono- and diiodotyrosine due to defect of the enzyme, deshalogenase. Hence excess iodine is lost in this way.

Many questions remain to be answered. First and foremost is the validity of Marine's hypothesis of the cycle: normal→hyperplasia→colloid goiter. Although there can be no doubt that iodine deficiency may lead to *hyperplastic goiter, the production of colloid goiter has yet to be achieved* in the laboratory. This is of paramount importance. One would like to know whether the morphologic alterations described so well by Aschoff continue to be encountered in endemic areas. In other words, what is the pathologic anatomy of endemic goiter today? The problem is certainly not as simple as the magic words "iodine deficiency" lead one to suspect. We have been too complacent about accepting theories concerning iodine deficiency and endemic goiter. It is therefore refreshing to read Greenwald's contributions[1181, 1182] and become aware that *the story is not as simple as one may* have thought.

problem The Public Health Service estimated a total of 25,000 cases for the year 1911. Because of the seriousness of the problem several special conferences were called The toxic theory was discussed and eliminated by the majority of observers in favor of the hypothesis that the disease was caused by a microorganism. Joseph Goldberger, who had a rich background in bacteriology and epidemiology as a result of his studies on yellow fever, typhoid, dengue, diphtheria, and typhus, was directed to undertake an investigation of pellagra. The story of pellagra from 1914 to 1929 is the biography of Joseph Goldberger

It did not take long for Goldberger to conclude that pellagra was not an infectious disease.[1184] He needed only to note that, although all the inmates of a Mississippi orphanage which he visited had pellagra, the employees never contracted the disease So he wrote at that early date, "the explanation of the peculiar exemption under discussion will be found in the opinion of the writer in a difference in diet of the two groups of residents." Goldberger, therefore, added meat and milk to the diets of the inmates of state institutions in Mississippi and Georgia [1185] Within a few weeks pellagra disappeared, not to show itself again

Goldberger's next step was to produce pellagra in healthy subjects by feeding them a deficient diet [1186] He persuaded the Governor of Mississippi to allow him to ask for volunteers from among the convicts at the State Penitentiary. Twelve agreed to submit to the test in return for their pardons. Eighty convicts served as controls, performing the same work but receiving a different diet The experiment lasted from April 19 to October 31, 1915 The dietary regimen was monotonous, though similar to that which was common all over the south at the time A typical day's menu (August 2, 1915) was as follows

BREAKFAST	Biscuits, fried mush, grits, brown gravy, coffee and sugar
DINNER	Cornbread, cabbage, sweet potatoes, grits and syrup
SUPPER	Fried mush, biscuits, rice, gravy, cane syrup, coffee and sugar

Five of the eleven men who completed the experiment developed what was considered to be pellagra The first cutaneous lesions were found on the scrotum Dermatitis of the exposed portions of the skin then appeared

Despite these experiments in human volunteers many students of the disease remained unconvinced and continued to look upon it as an infection. Goldberger [1187] was forced to go to what today seem like needless extremes in order to convince all the doubtful that the disease was nutritional in origin and not infectious in nature He attempted to transmit the

hunger edema in its historical perspective and points out that, although some good descriptions can be found in the literature of the 19th century and before, there are not many in all, especially when one considers the great wealth of information available concerning famines in general. To explain this McCance suggests that the metabolic effects of food shortages were masked by the more dramatic ravages of the great epidemic diseases which always accompanied famines until the first decades of this century.

Following the first world war, edema became prevalent in Central Europe where undernutrition was widespread and prolonged. A good deal of attention was focused on this subject. Many clinical observations were reported and some attempts were made to produce the syndrome experimentally during the ensuing decade. By the 1930's it was clear that hypoproteinemia, or, more precisely, hypoalbuminemia, could result from poor nutrition and, in turn, could give rise to edema. In fact, up until the early 1940's, it was believed that the greatest contributory factor to nutritional or hunger edema was reduction in plasma proteins. A number of studies cited by McCance,[1100] Gounelle[1097] and Keys *et al.*[12] served to indicate that the problem was far more complex than had been believed and that a number of variables had to be considered: (1) generalized increase in body fluid which in part replaces lost fat and cells; (2) salt intake; (3) posture, (4) tissue tension, (5) vascular factors; (6) renal function, and (7) hormonal effects. We cannot discuss all of these in detail. Listing them serves to indicate that the problem of the pathogenesis of hunger edema in a given case is a formidable one and is not simply the result of hypoproteinemia. For it is now clear enough that, though plasma proteins may be decreased, edema may not necessarily be present; so, too, serum albumin concentrations may be within normal limits, and edema can be well-marked.

THE PELLAGRA SYNDROME

The first accurate description of the disease, "mal de la Rosa," was written by Casal in 1735[1183] though his work was not published for some years. In his *Historia natural, y Medica del principado de Asturias*, the dermatitis, with its prominent distribution about the neck, the sore mouth, the gastrointestinal disturbances, and the mania, which may terminate the disease, all are described Frapolli introduced the name pellagra (*pelle agra*, rough skin) in 1771.[1183] Even at that time and during the ensuing years, the prevalence of the disease in such countries as Spain, Italy, Roumania and France, where maize formed a considerable part of the diet, made certain [illegible], in which some toxic material

[illegible] ith explosive violence in the southern part of the United States and immediately became a national

cured for a time by the administration of saline solution alone.[1210] Further studies in dogs,[1213] rats,[1214] and swine[755, 1215] indicated that on a corn or low-protein diet nicotinic acid was necessary to promote weight gain On a high-protein (casein) diet the nicotinic acid requirement is reduced to nothing Finally, twenty-three years after Tanner's clinical observation noted above, tryptophan was shown to be a precursor of nicotinic acid

As a further complication, the possible presence of a pellagragenic factor in corn[756] must continue to be considered, having not been entirely proved or disproved

Clinically, pellagra manifests itself by skin lesions, by functional and anatomical changes in the alimentary tract and by varied disturbances of the nervous system[1093]

The skin changes have been classified by Smith and Ruffin[1194] as. (1) those on the exposed surfaces of the body which are precipitated by exposure to sunlight, (2) "dyssebacia" or plugging of the sebaceous glands about the alae nasi, (3) hyperkeratosis over the bony prominences, (4) excoriated lesions of the skin about the perineum, scrotum and female genitalia, (5) fissures of the lips (cheilosis) and cracks at the angles of the mouth (angular stomatitis), and (6) lesions of the external portions of the eyes.

The skin changes begin as areas of erythema which may burn or itch As the lesions progress the skin becomes swollen, tender and more inflammed Vesicles and bullae form and then usually rupture Desquamation begins in the central portion of the lesion which by this time has become darkened and scaling Hyperpigmentation may be extremely prominent Lesions about the genitalia and in the vagina have the same evolution Scaling lesions may develop without erythma and vesiculation

Microscopically[1193, 1194] the earliest change is rarefaction of the superficial portion of the corium with dilitation of the blood vessels and proliferation of their endothelial lining cells This external portion of the corium seems to have a spongy appearance Next, the epithelium, which has begun to show hyperkeratosis, separates from the corium with the formation of vesicles filled with fluid containing fibrin, red blood cells, and melanin pigment Vesiculation may begin intraepidermally or subepidermally With rupture of the vesicles capillary proliferation occurs Ulcerated areas are usually covered with fibrin and leukocytes Relatively little secondary infection is found, however Healing takes place in the usual fashion. Microscopically, the skin over the bony prominences shows hyperkeratosis with perhaps some increase in inflammatory cells in the superficial portions of the corium

As already noted, involvement of the lips is common.[1192] The cheilosis consists of reddening and thinning of the exposed surfaces. Glossitis and

disease to himself, to his wife and to his fellow workers by injecting them with the blood from pellagrous patients, by swabbing their nasopharyngeal tissues with secretions from pellagrins, and by innoculating them with skin scales and excreta from the patients. All such attempts failed

During this period Goldberger and his collaborators had begun to collect data concerning the typical dietaries of the south, seasonal variations in the disease, and other factors It was clear to him that meat and milk were preventive or curative; yet these were expensive foodstuffs. Something cheaper had to be found. In 1922, he [1188] suggested that amino acid deficiency was "probably the etiological factor in pellagra," having excluded minerals and the then known vitamins. His associate, Tanner, had noted the dramatic changes produced in a pellagrin following the administration of tryptophan which "surpassed anything I have ever seen in a case of pellagra in an equal period of time." [1189]

Goldberger had also become impressed with the similarity of the blacktongue syndrome in dogs (page 329) to pellagra.[1200, 1201] An experimental animal for the study of pellagra appeared to be at hand. Shortly thereafter, when yeast was found to be effective in curing or preventing blacktongue, Goldberger went on to show that the pellagra preventive or P-P factor was heat stable and different from the heat sensitive portion of vitamin B.[1094] Large numbers of foodstuffs such as liver, yeast, et cetera, had been tested for their pellagra preventive value.

By 1930, a year after Goldberger's untimely death, the problem of prevention and cure of pellagra was in theory, at least, settled. A few years more were to elapse until the efficacy of nicotinic acid in the treatment of blacktongue was reported.[850] Late in 1937 and early in the following year the effectiveness of nicotinic acid in the treatment of pellagra was clearly demonstrated [1190, 1191]

For a while the case seemed closed. Yet, for serious students of pellagra certain questions had not been answered. So, too, studies of nicotinic acid-deficient diets in experimental animals raised other perplexing problems (page 331). The role of sunlight in precipitating the naturally occurring disease had been insisted upon by Frapolli from the very beginning [1183] This phenomenon was studied in some detail by Smith and Ruffin [1192] in pellagrins who were first observed on the ward under controlled conditions and then exposed to the direct rays of the sun for varying periods of time. Not all the patients reacted but some (15 out of 35) developed dermatitis and constitutional symptoms, or even glossitis, diarrhea and dementia. At the present time the role of radiation in the pathogenesis of pellagra is recognized but not well-understood.

When dogs were placed on a nicotinic acid-deficient diet, the blacktongue syndrome (page 329) promptly appeared, but the disease could be

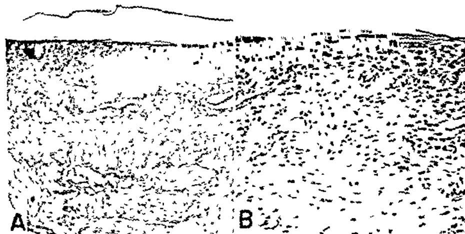


FIGURE 121 PELLAGRA

Tongue (J H H 8847) A and B, low (x 14) and higher (x 35), magnifications of lingual epithelium to show atrophy and chronic inflammatory reaction beneath Compare with normal, same magnifications, Figure 174

stomatitis appear early in the course of the disease, manifesting themselves first by pain and redness The process begins on the inner aspect of the lips and along the *lateral margins of the tongue Such areas may ulcerate* and become infected, particularly with Vincent's organisms, a phenomenon which is especially likely to happen as the lesions are spreading to involve the dorsum of the tongue If the disease is in a chronic stage, the filiform and fungiform papillae become atrophic, hence, the tongue assumes a *smooth appearance Pains in the esophagus and the stomach are common* Diarrhea is usually present So, too, nausea and vomiting may be prominent

Microscopic examination of the tongue reveals atrophy of the epithelial papillae with a superficial acute and subacute inflammatory reaction Ulcerations of the esophagus and stomach are non-specific, though in the *former area secondary involvement by Vincent's organisms may be prominent* The small intestine rarely shows very much grossly or microscopically On the other hand in the colon, "changes are remarkably constant and are all but specific," according to Denton [1124] Numerous small ulcers are found, covered by a fibrinous membrane Larger ulcerations have overhanging margins Small abscesses are seen in the submucosa *Cystic dilatations of* the mucous glands are prominent, these are reminiscent of what has been described in pantothenic acid deficiency in swine (page 228) Fatty liver, usually periportal in nature, is seen.

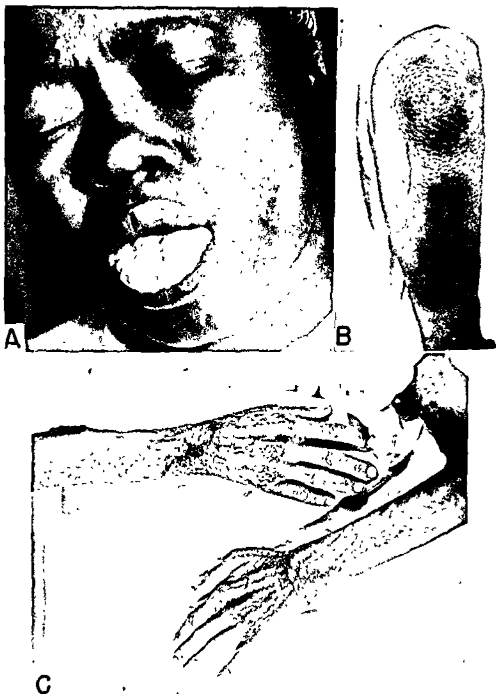


FIGURE 120 PELLAGRA

Tongue. A. Smooth "magenta" tongue B Skin Hyperkeratosis, elbow In addition, some excess pigmentation is present. C. Classical, scaling, hyperpigmented, dermatitis of forearms and hands. (Courtesy of Dr. T. D. Spies)



FIGURE 123 PELLAGRA

Skin (J H H 8847) *A* Low power (x 7½) of vesicular lesion found at autopsy which shows separation of epithelium from corium *B* and *C* (x 35) Edges of vesicle to indicate plane of separation A thirty-three-year-old female who was admitted to the hospital with soreness and burning of arms, having eaten poorly for several months She died in 1928 without any specific therapy

←

nausea, vomiting and anorexia Diet had been inadequate Lesions on the hands followed prolonged exposure to a baking oven. He was given fluids and a basal diet containing no B vitamins for eight days without improvement On the ninth day 90 mgm of nicotinic acid were administered intravenously There was a steady improvement of the tongue within twenty-four hours, two weeks later the hands appeared as in *B* and exposure to a heat lamp failed to provoke a relapse (Courtesy of Dr D T. Smith and *The Southern Medical Journal*)

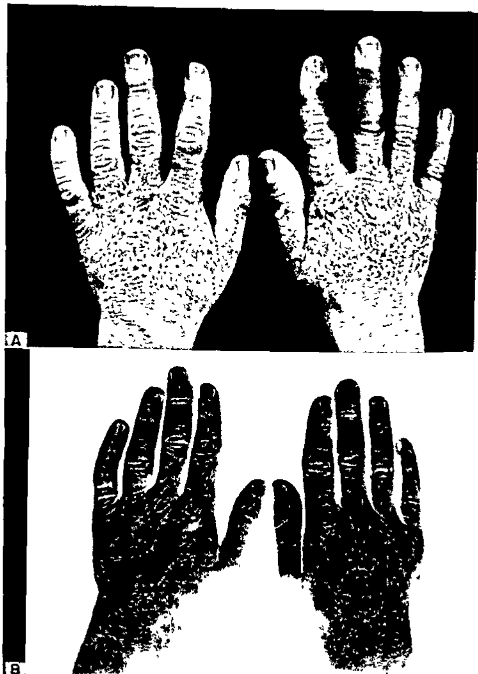


FIGURE 122 PELLAGRA.

Skin A This fifty-four-year-old white male was admitted to the hospital with an erythematous scaling over the dorsum of the hands, diarrhea, sore mouth and tongue,

→


A


B


C

←

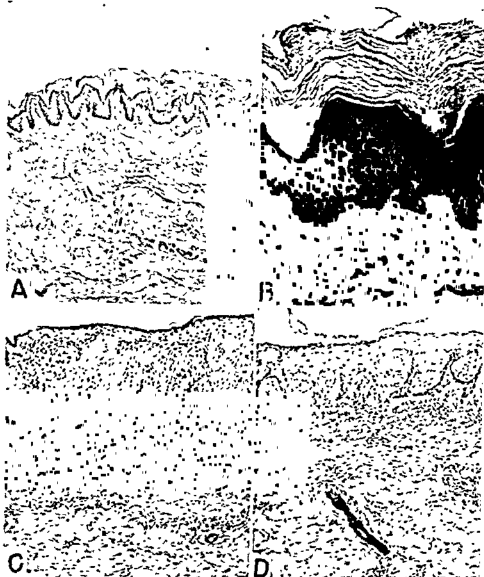


FIGURE 124 PELLAGRA

Skin *A* ar ... twenty-two ye
old female ... (1928) witho
specific thei ... dermatitis.

FIGURE 125 PELLAGRA


A


B


C

←

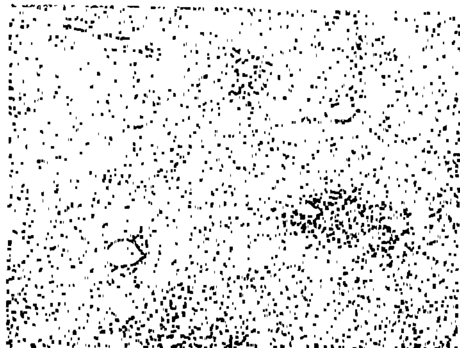


FIGURE 126 PELLAGRA.

Liver. (J H H 12860) Fatty infiltration in an alcoholic who had developed pellagra. Virtually no treatment had been administered before death (1931) The fatty change is predominately periportal (× 35).

Clinically symptoms and signs of involvement of the nervous system in pellagra are extremely variable.[1093, 1195, 1199] All types of mental abnormality may be encountered: melancholia with suicidal tendencies, excitement, stupor, mutism, and delerium. Epilipsy and severe headaches are described Dimunition in thermal, tactile and painful sensibility is seen. So, too, ataxia, tremor, sometimes of the intention type, dysphagia, dysarthria and reflex changes occur.

At autopsy, there is evidence of damage to many neurons, to the peripheral nerves and to the fibers in the spinal cord and, to a lesser extent, those in the brain In addition, changes in the small vessels of the brain are found Thus, the entire nervous system appears to suffer a diffuse insult.[1196, 1197, 1198, 1287 1288]

The neurons show an increase in pigment and in the fat content of their cytoplasm. These materials tend to accumulate in the central part of the cell, displacing the nucleus to the periphery The basophilic, metachromatic Nissl substance is also displaced toward the cell membrane In severely damaged cells this ribonucleoprotein undergoes lysis The pattern of in-

volvement of the neurons is variable. Usually, however, the sensory groups are more severely affected than the motor cells. Particularly susceptible are the dorsal root neurons and the cells of Clarke's columns.

Lesions in the peripheral nerves and spinal nerve roots are evidenced by the presence of free fat as a result of myelin degeneration. Similar foci are found in the white matter of the spinal cord where both lateral and dorsal columns may be involved. The early changes are patchy and focal. Chronic progressive changes give rise to tract degenerations. Neutral fat may be found in phagocytes about blood vessels in the white matter of the brain. Large lesions in this region are uncommon. In the areas where nerve fiber degeneration has occurred, gliosis is found. Vascular changes also appear to be part of the changes in the nervous tissues, though they have not been observed in all cases of pellagra. When present they consist of lipoid infiltration and pigmentation of the small cerebral arterioles and capillaries with hyaline deposits in the walls of the latter.

The fact that classical pellagra has occurred endemically in areas where

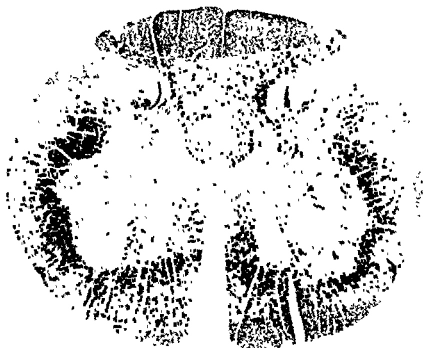


FIGURE 127. PELLAGRA.

Spinal cord. (J.H.H. 11663.) This autopsy has been reported by Langworthy [illegible]



FIGURE 128. PELLAGRA.

Spinal cord, human *A* Areas of demyelinization of spinal cord of individual dying of pellagra (J.H.H, 11663). *B* and *C*. Changes in neurons from same case to show chromatolytic alterations.

corn forms a large part of the diet has made it clear to many that the disease must be looked upon as a multiple deficiency syndrome. The deficiencies of corn have been discussed elsewhere (page 15). It is only necessary to point out here that a number of the pathologic changes which go to make up the pellagra syndrome are not necessarily produced exclusively by nicotinic acid (tryptophan) deficiency. For instance, typical glossitis has been observed in the human as a result of a lack of the following nutrients: iron (page 229), pyridoxine (page 248), riboflavin (page 218) and biotin (page 266). Scrotal dermatitis may be related to riboflavin deficiency (page 218). Cheilosis may respond to pyridoxine therapy.[1511] Scaling dermatitis has been seen in patients subjected to pyridoxine deficiency (page 248). It is virtually impossible today to characterize specifically the exact deficiency which may exist in a given case of pellagra in a particular part of the world. This is a serious defect in our understanding of deficiency disease that should be remedied. If one asks the questions: to what biochemical defects are the tongue, lip, alimentary, skin, neurological, and other lesions due, the answer is, "we don't know." Perhaps some day we shall, though it is likely that the syndrome will have to be studied in the experimental animal more extensively than it has in the past.

THE BLACKTONGUE SYNDROME

A disease in dogs, which clinically is characterized by vomiting, loss of appetite, and diarrhea, and in which at autopsy congestion and ulceration of the gastrointestinal tract are found, was first recognized in Germany during the latter part of the last century.[1200] In America this canine malady was called blacktongue and was regarded as infectious in nature, although attempts at demonstrating an etiologic agent were unsuccessful. According to Joseph Goldberger,[1201] blacktongue was well-recognized by veterinarians in the southern part of this country during the early part of the century. Since pellagra was so prevalent in the same area at this time, the close resemblance of the two diseases was striking. Goldberger[1201] quoted Dr. Spector of Concord, N.C., who stated in a brief note, "so-called blacktongue is canine pellagra."

The first experimental evidence for the nutritional origin of blacktongue appeared in 1917, when Chittenden and Underhill[1202] described a syndrome which they had observed in dogs which were placed on a diet of dried peas, cracker meal, and cotton seed oil or lard, with or without the addition of small amounts of meat. They wrote, "The onset of pathological symptoms is generally very sudden. Usually the first abnormal manifestation is a refusal to eat, and examination will reveal nothing to account for the loss of appetite. The animal lies quietly in its pen and is apathetic. After continued refusal to eat for a day or two, the mouth of the dog will present a peculiar

and characteristic appearance. The inner surface of the cheeks and lips and the edges of the tongue are so covered with pustules as to give the impression of a mass of rotten flesh. The odor from these tissues is foul and almost unbearable. When stroked with absorbent cotton the mucous lining of the mouth comes away in shreds. Intense salivation is present. The teeth appear to be solid and normal. A bloody diarrhea is present, attempts at defecation being very frequent and resulting in the passage of little more than a bloody fluid of foul odor. In some cases, the thorax and upper part of the abdomen may contain many pustules half an inch in diameter which are filled with pus organisms. No other skin lesions are prominent. Death usually results without any particularly striking features.

"At autopsy two types of conditions are recognizable. In the animals presenting foul mouth and bloody diarrhea the chief interest centers in the lower bowel and rectum which exhibit an intense hemorrhagic appearance. With those animals dying rapidly from convulsions the only visible abnormality of the alimentary tract is the presence in the duodenum of one or more large ulcers"

It was concluded: "In the essential features, the pathological manifestations described in this investigation closely resemble those which may be observed in human pellagra."

At the time that this report of Chittenden and Underhill appeared, Goldberger had already succeeded in producing experimental pellagra in the human [1186] He and Wheeler modified their human diet to one consisting of cornmeal, peas, casein, cod liver oil, cottonseed oil and salts [1201] By this means a clinical syndrome was produced in dogs which appeared identical with that which the New Haven investigators had described. However, meat or yeast was found to be effective in preventing the disease. This was, of course, not consistent with the results of meat feeding by Chittenden and Underhill.

The pathologic changes occurring in the tissues of Goldberger's dogs were studied by Denton,[1203] who found microscopic lesions in the mucous membranes of the mouth, pharynx, esophagus, and intestines, and the skin of the scrotum The underlying change was interpreted as a "degenerative process affecting the superficial connective tissue of the mucous and dermal membranes Changes in the supporting tissues of these mucoid membranes are followed by secondary ones in the epithelium. The lesions tend to terminate in an extensively necrotic and diphtheritic inflammation of the upper alimentary tract." Denton likened the changes in the dogs to similar lesions which he had described previously in autopsies on pellagrins.[1194]

Soon, a second report, which was an extension of the earlier experiment, appeared from the Yale laboratory, this time by Underhill and Mendel.[1204] They confirmed that the diet of peas, cracker meal and cotton seed oil

would produce the syndrome described 10 years before. Tissues of their animals were studied in some detail by R A Lambert, who called attention, in particular, to "hyaline degeneration and swelling of the subepithelial connective tissue" of the buccal lesions The final conclusions of the New Haven laboratory were that the most effective protective substances appeared to reside in fresh pig liver, butter fat and, in particular "crystallized carotin"

These studies by the Yale investigators and by Goldberger and his co-workers at the U S Hygienic Laboratory were confusing since an identical syndrome appeared to have been produced in dogs which would respond on the one hand to liver or carotin and on the other to meat or yeast

Similar studies in which a Goldberger-type diet was utilized were carried forward at the Rockefeller Institute, where Rhoads, *et al* [1205, 1206] showed that severe anemia might accompany the chronic stomatitis, salivation, glossitis, loss of weight, and diarrhea which characterize the blacktongue syndrome Moreover, leukopenia and alterations in the bone marrow which were reminiscent of those seen in the sprue syndrome or pernicious anemia were observed Lesions in the nervous tissues were also described

At the same time, Smith and his associates were studying the blacktongue syndrome at Duke University [1207] They were able to reproduce both types of blacktongue and to cure the Chittenden-Underhill form with cod liver oil, probably due to its vitamin A content, and the Goldberger type with meat or yeast The buccal flora was studied by these investigators In both types of blacktongue, the oral lesions appeared to be the result of infection with the fusospirochetal group of organisms, which were considered secondary invaders as a result of lowered tissue resistance

Finally, important observations of the Wisconsin group seemed to conclude the blacktongue story, though, as will be seen, not for long Elvehjem and his co-workers had been studying the effects of certain liver fractions on blacktongue produced by a modified Goldberger diet [1208] They were soon able to show that the factor necessary to cure and prevent blacktongue was nicotinic acid amide They found that dogs could be maintained in health on the basal blacktongue-producing diet with added nicotinic acid or its amide for several months [850, 1209]

The matter was not so easily settled, however, since several facts led Handler and his associates to question, and properly so, the role of nicotinic acid in the blacktongue syndrome In the first place, no significant differences could be detected between the cozymase content of certain tissues of normal dogs and animals succumbing with the manifestations of blacktongue [851] Furthermore, animals which were about to die of typical blacktongue could be saved by the parenteral administration of salt solution. This effect did not appear to result from a replacement of fluid which

had been lost from the gastro-intestinal tract as a result of diarrhea, since some animals never exhibited frequent stools.[1210] The administration of salt solution prolonged life in some of the animals for as long as 180 days However, though all dogs finally succumbed, the clinical course was somewhat different from that of typical blacktongue. Furthermore, Handler demonstrated that, although the blacktongue syndrome can be produced with ease when the classical Goldberger cornmeal diet is fed, purified rations which contain even less nicotinic acid might not provoke the disease [1211]

At *the same time, workers at Wisconsin*[1212] *had been feeding purified* diets containing 19 per cent protein to weanling puppies and had observed extensive loss of weight, anorexia, inflammation of the gums, and erythema of the palate after fourteen to eighteen days Similar changes could be produced in adult dogs after thirty to forty-five days; however, the alterations were not entirely characteristic of blacktongue. Inasmuch as such deficient animals would not consistently respond to nicotinic acid therapy, the ration was supplemented with a folic acid concentrate, derived from solublized liver extract. The dogs were then found to respond uniformly to nicotinic acid therapy; furthermore, they did not relapse. Dogs deficient in nicotinic acid but receiving adequate folic acid appeared to have a lower incidence of buccal lesions; such might indicate that this part of the blacktongue syndrome is not due to nicotinic acid deficiency.[853] Inconclusive studies of the blood were reported in these two groups of experiments; this is unfortunate since Handler had shown that dogs on a cornmeal ration developed a progressive anemia which in some animals was macrocytic in character.[1213] He proposed the hypothesis that decreased red blood cell formation results from an inadequate supply of cozymase, which is needed for the respiration of the immature erythrocyte.

Finally, the importance of corn in the pathogenesis of blacktongue because of its low tryptophan content and hence its insufficiency as a nicotinic acid precursor has been well-established.[1214, 1215] On the other hand evidence for a "pellagragenic factor" in corn has been presented[856] but needs further investigation.

At the present time the pathogenesis of the blacktongue syndrome has not been further clarified. A good deal more needs to be learned of the biochemical as well as the anatomical alterations which give rise to this entity. From the brief history which has just been related, it appears that the Goldberger-type diet is deficient in tryptophan, and hence in nicotinic acid. What effect lysine supplements to the corn might have is something which remains to be studied. So, too, additions of vitamin B_{12} and other nutrients to the diet should be evaluated. It may be significant that the intestinal changes described in blacktongue resemble those which are found in swine

which have been made deficient in pantothenic acid The role of folic acid in curing the granulocytopenia is important to evaluate, since a reduction in white blood cells might be responsible for the invasion of the buccal tisues by microorganisms. Moreover, the therapeutic effects of saline are most puzzling. The original blacktongue syndrome, which was first described by Chittenden and Underhill, needs to be clarified. Although vitamin A deficiency appears to be the most likely explanation, such may not be the entire story The response of both types of blacktongue to antibiotics would seem worthy of investigation All in all, after reviewing the fascinating story of twenty-five years research on blacktongue, 1917-42, it is perfectly obvious that much might be learned if all was to be repeated by one laboratory, employing both natural and synthetic diets, studying biochemical and structural alterations, and investigating the effects of various therapeutic agents

KWASHIORKOR

The title of this section is kwashiorkor, but could just as well be, *nutritional edema of infancy, syndrome of malignant malnutrition, sindrome poliocarencial infantil, infantile pellagra, Mehlnahrschaden, fatty liver disease*, or a host of other names. Today, most workers in the field of protein malnutrition appear to favor the term *kwashiorkor* What does this word mean and how may one define this clinical and pathological entity?

The first description of the prominent features of the disease appeared in 1933 when Cicely Williams reported a series of cases from the Children's Hospital at Accra [1216] "The syndrome consists of oedema, chiefly of the hands and feet, followed by wasting, diarrhoea; irritability, sores, chiefly of the mucous membranes, and desquamation of areas of the skin in a manner and distribution which is constant and unique. The disease attacks children of either sex, between one and four years old It appears to be due to some dietetic deficiency and to be uniformly fatal unless treated early. In all cases seen there was a history of an abnormal diet Breast feeding had been given by an old or else a pregnant woman, and the only supplementary food consisted of a preparation of maize Three post-mortem examinations have been held. All three have been exactly like those quoted at length in case 2, the only thing of note being a pale, fatty, almost diffluent liver"

Two years later Williams contributed a second paper, *Kwashiorkor A nutritional disease of children associated with a maize diet.*[1217] This is the first time that the word kwashiorkor was used Williams indicated that this term was in use on the Gold Coast to refer to the altered physiological state of a child, usually the first, which developed after weaning, because of the expectation of a new baby In other words "kwashiorkor indicates the disease the deposed baby gets when the next one is born." It would not

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appear to be correct to translate kwashiorkor to mean "red boy" or some other term.

At the same time the disease had begun to be recognized and written about in many other areas: Latin America, Mexico, South America, Jamaica, southern India, and Africa from the southern border of the Sahara desert to the northern boundaries of the Union of South Africa. Moreover, it became clear that *kwashiorkor* is related to the *starch dystrophy syndrome* or *Mehlnahrschaden*[1574] described in Europe during the first part of this century

In her initial description of the syndrome, Williams[1216] pointed out the curious tribal customs of the natives of the Gold Coast, where babies are fed on the breast until the next child is born. The breast feeding period is rarely less than two years and may last longer. After weaning, which may be intermittent as a result of foster breast feeding, the child receives a diet consisting almost exclusively of corn in the form of a paste or gruel. The adult diet is poor, too, composed mainly of maize supplemented by yams and cassava, with only small amounts of fish and meat. No milk or butter is used. Occasional fruit is available and tomatoes, onions, eggplant and okra are fairly widely consumed. Thus, the infant, or more usually the two to three year old child, is weaned from a diet of milk, which comes from a poorly nourished real or foster mother, to a diet which consists virtually exclusively of corn

Since kwashiorkor is found in so many areas one might expect its clinical and pathological manifestations to vary somewhat from place to place depending on differences in local customs and in diets. This is true. The following general account is derived from a group of well-documented studies which deal with the disease in Africa,[1218, 1219] Central America,[1220, 1221] Brazil,[1222] Jamaica,[1223] India,[1224] and certain other tropical and subtropical areas [1225, 1226]

There would appear to be agreement that the clinical syndrome usually consists of the following: (1) Retardation of growth; (2) Weight loss with muscular wasting; (3) Apathy and other psychic changes, (4) Dermatitis, *(5) Changes in the hair; (6) Edema of variable degree; (7) Diarrhea,* and (8) Enlargement of the liver.

Soon after weaning the child who may be expected to develop kwashiorkor evinces retardation in growth This failure is manifested specifically by decrease in length with respect to age and by a reduction in body weight, provided, of course, that excessive amounts of water are not being retained. When edema is present, appearances may be extremely deceptive. Among patients in some areas, such as Jamaica, the amount of subcutaneous fat may not be decreased, presumably as a result of the child's high carbohydrate intake[1223] When x-ray studies of the skeleton are car-

ried out, several abnormalities may be encountered.[1227] A definite decrease in bone age is found, that is the number of centers of ossification, for instance in the hand, is lower than one would expect to find in a well-nourished child the same age. This would indicate that the deficient state has been in progress for some time. In addition, dramatic effects of more severe episodes of growth arrest are seen in the form of transverse strata in the metaphyses of long bones. These alterations may be interpreted to arise because growth of epiphyseal cartilages has virtually ceased for intervals during which osteoblasts continued to deposit bone on their under surfaces.[1228] With resumption of the growth of cartilage these strata, or transverse lines, are left as markers as the epiphyses move away from them.

The psychologic aspect of the clinical pattern of kwashiorkor has aptly been summarized as a state of "peevish mental apathy,"[1225] which has been described more fully as follows "A child with kwashiorkor is dull, apathetic, and miserable. It rarely screams or cries, a low miserable whimper is the only vocal sign of its wretchedness. We are all familiar with the African child who, terrified by the European doctor, fights and resists examination to the limit of its strength. Not so the kwashiorkor child, it will rarely if ever resist examination in the least degree, and will never fight and scream, its apathy is too great. Children with kwashiorkor are so dull and apathetic that if put to sit in one place they will remain sitting there until lifted up again. *They never, as do so many other children, go wandering off down* the ward to investigate matters for themselves. If one can get a smile out of a child with kwashiorkor, one can assume it is well on the way to being cured."[1229]

Changes in the skin and at least one of its appendages, the hair, have received a prominent place in clinical descriptions of kwashiorkor. The skin changes include atrophy and areas of hyperkeratosis with excessive pigment formation. On the other hand, generalized loss of pigment may be seen. So, too, following healing of skin lesions, localized foci of hypopigmentation may be encountered. The skin lesions appear to have somewhat the same evolution as those of pellagra erythema, vesiculation, scaling, hyperpigmentation. They differ, however, from pellagra in that they do not appear to be affected by sunlight and consequently do not have the same distribution. The dermatitis of kwashiorkor is seen most prominently over the trunk, thighs, buttocks, inguinal regions and popliteal spaces. Sometimes the skin lesions have the appearance of second degree burns. Hyperkeratotic changes give a flaky appearance to the skin, this has been called the "flaky-paint" dermatosis.

When changes are seen in the hair, they are striking, though other causes of hypochromotrichia must be excluded. If the hair is black it may become lighter in color, often acquiring a reddish tint. A dramatic characteristic is



FIGURE 129 KWASHIORKOR

Hair. Zone of loss of pigment of hair in child who has recovered from kwashiorkor This is the *signo de bandera*. (Courtesy of Dr. Arnold Schaefer)

that the loss of color may be sharply demarcated, hence, a clearcut band of depigmentation is seen (*signo de bandera*). This may be even more dramatically brought out following therapy. The hair may be thin, dry and sparse and may be readily pulled out. In African infants the hair may be straight, distinctly an abnormal feature.

Lesions of the mucous membranes and the mucocutaneous junctions are common The glossitis, cheilosis, and angular stomatis are similar to these changes seen in other deficiency disease syndromes (page 452).

Edema is usually noted first in the feet and lower legs. Swelling then appears in the hands, thighs, sacral region, back, upper extremities and face. Ascites is a late manifestation of the disease.

Among the evidences of gastrointestinal disturbance anorexia and vomiting are common. Diarrhea is usual. The stools contain an excessive amount of fat. The diarrhea appears to have several causes: (a) diminution of pan-

creatic secretions; (b) loss of absorption of intestinal contents due to changes in the mucosal cells (see below), (c) bacterial infection,[1230] and (d) parasitic infestations.[1231] More will be said later about the relation of animate agents to the exacerbations of the disease

On palpation the liver is usually found to be enlarged and may extend several fingers breadth below the costal margin The spleen is ordinarily not enlarged.

Anemia[1232] is commonly, though not invariably, present. This may be related to deficiencies of nutrients such as iron or to intestinal parasites

A large number of biochemical studies have been carried out on blood, intestinal contents, urine and tissues of children in various stages of kwashiorkor as it progresses to death or during the recovery period following treatment.[1233 1234]

The most consistent alteration in the blood, one of the changes upon which the diagnosis of the disease rests, is hypoproteinemia The fall in serum protein level is due to a reduction in the albumin concentration which may be extreme Generally a rise in the total globulin is observed, of the globulins, the alpha 1 and gamma fractions are increased.

Alterations in the mineral constituents of serum have been inconspicuous[1248] However, decreases in certain vitamins have been observed, these include vitamin A (and carotene), vitamin E, thiamine and riboflavin A decrease in serum cholesterol levels has been reported. The values of certain enzymes in the blood are reduced, alkaline phosphatase, amylase, lipase, and esterase are all low and rise following therapy

The concentrations of certain enzymes in duodenal secretions have been studied Low values have been found for lipase, esterase and trypsin The fat content of the intestinal contents is elevated Fat balance studies have revealed a marked deficit[1235]

The urinary excretion of amino acids has been reported on[1236] Preliminary studies would indicate that free aminoaciduria is absent and may appear during the "recovery syndrome" Certain abnormalities are seen in the amino acid excretion pattern. a high ratio of isoleucine to leucine, a high ratio of phenylalanine to tyrosine, and a low excretion of threonine. Whether future studies will continue to confirm these abnormalities remains to be seen.

Chemical analyses of tissues removed by biopsy have revealed a number of changes. The fat content of the liver is increased This rise is due to neutral fat as well as to cholesterol For instance, in a series of Jamaican infants the total fat content on a wet weight basis was found to be 33 8 per cent, after therapy the fall to the normal of 3 1 per cent.[1237] A decrease in cytoplasmic protein has also been noted since the RNA content is low in relation to DNA; the former rises following therapy.[1237]

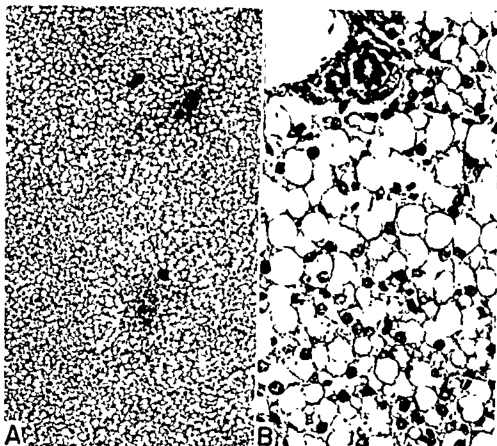


FIGURE 130. KWASHIORKOR

Liver *A* (x 100), and *B* (x 450). Diffuse fatty infiltration of the liver in child dying of kwashiorkor. (Courtesy of Dr. J. N. P. Davies.)

The activities of certain enzymes in tissues have likewise been studied. Pseudo-cholinesterase activity of liver biopsy tissue is reduced. So, too, the vitamin content of liver tissue may be reduced; particularly depressed are vitamin A and niacin levels.

The findings which have been described in autopsies on children dying of kwashiorkor are rather characteristic and help corroborate as well as amplify the clinical findings which have already been described. Edema may be variable and will, of course, depend to some extent on preceding treatment. Anasarca is the rule, however, the classical case has much fluid in the subcutaneous tissues, peritoneal, pleural and pericardial cavities and in the retroperitoneal area. Ascites is particularly prominent if vascular disease is present in the liver.

The liver may appear markedly enlarged or may not be particularly changed in volume.[1238] The color varies depending on the amount of fat present. Those with the most lipid have an ochre or canary-yellow appearance. The capsule is shiny and tense, particularly when a large amount of lipid is present. On microscopic examination, varying amounts of fat are found in the liver lobules From studies at autopsy in Africa and biopsy observations during the return of the liver to normal after the institution of appropriate therapeutic measures it would appear that fatty infiltration begins in the periportal areas Here fine droplets are found in the cytoplasm. These droplets distend the cell so that the sinusoids appear to be compressed. As the process continues the fat begins to accumulate in the cells of the mid-zonal portion of the lobule and finally comes to occupy all of the cells about the central vein By this time the organ has become so infiltrated with fat that it is sometimes difficult to recognize as liver tissue, this can only be determined by the presence of the periportal triad of artery, vein, and bile duct. In the periportal areas variable numbers of cells are found infiltrating the connective tissues, these are preponderantly mononuclear elements Following therapy, fat first disappears from the central part of the lobule, then the mid-zonal, and finally the periportal cells are restored to normal

Most pathologists who have studied the livers of children dying from kwashiorkor have found little else In Africa one may find malarial pigment and the presence of certain parasites such as ankylostomata and schistosomes Parasites may also be found when the liver is examined in other geographical areas In general it can be stated that relatively little increase in connective tissue is found in the fatty liver Such a finding is to be expected when it is realized that kwashiorkor is ordinarily an acute disease and if treatment is not instituted the child will die Hence if fibrosis might be anticipated there is not much time for proliferation of excessive collagen fibers to occur. Davies[1238] has described an increase in reticulum fibers in the periportal areas, Tejada[1221] has pictured similar alterations Unless some complicating factor is introduced no further connective tissue proliferation appears during the period the disease runs its course in the ordinary, untreated case

The chemical evidence of increased fat content in the liver has already been mentioned. Histochemical studies of liver tissue obtained at biopsy have revealed an increase in alkaline phosphatase activity.[1239]

In addition to the fatty infiltration another alteration has been observed in the liver of infants dying in Jamaica[1223, 1240] and India. This change consists grossly of a gelatinous thickening of the portal vein radicles in the liver. On microscopic examination extensive intimal proliferation is found in the large and small portal veins, so that eventually many of these vessels

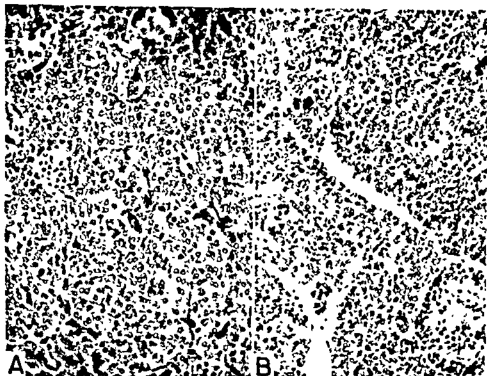


FIGURE 131. KWASHIORKOR.

Pancreas. *A* Normal (x 250). *B* Ascinar tissue from child dying of kwashiorkor. Note extreme atrophy (x 250). (Courtesy of Dr. J. N. P. Davies)

become occluded. This alteration, which has been called venous occlusive disease (V.O.D.), gives rise to a scarred liver; ultimately death occurs from liver failure The alteration has been shown to be due to the practice of giving infants infusions of bush tea containing the senecio leaf.[1226] Such lesions are seen in adults[1241] and have been well-authenticated in the experimental animal.[1242]

A good deal has been made of pancreatic changes in kwashiorkor, particularly by Davies.[1238] The earliest alteration is a reduction in size of the ascinar cells with a corresponding diminution in the number of secretory granules. This is followed by hyalinization of the cells and dilatation of the small ducts Coincident with these lesions new connective tissue begins to appear about groups of ascinar cells and around the ducts. Alterations of the islets of Langerhans are not striking until the connective tissue becomes excessive. An important question, which has been raised concern- [illegible]



FIGURE 132 KWASHIORKOR

Stomach *A* (×55), *B* (×145) and *C* (×400) Atrophy and cystic dilatation of glands in child dying with kwashiorkor (Courtesy of Dr J N P Davies)

can be settled only in the experimental animal, since virtually all cases of kwashiorkor now coming to autopsy have been treated

Renal lesions, consisting of hyalinization of glomeruli and proliferation of the capsular epithelial cells have been described at autopsy on infants dying of kwashiorkor [1238] Whether these represent specific alterations or not remains to be settled [1243]

Changes which have been observed in other organs are: atrophy of the glands of the intestinal mucosa, atrophy with decrease in colloid and fibrosis of the thyroid tissue and atrophy and loss of lipid from the adrenal cortex and atrophy of the salivary glands.

How may one reconstruct the natural history of kwashiorkor? To do so one must examine the background, nutritional and other, of both mother and infant There can be no doubt that the maternal organism is poorly nourished; frequently calories are deficient but more important the protein content of the diet is poor, quantitatively and qualitatively. Moreover, the amounts of certain inorganic elements such as iron and iodine may be low. In addition, the presence of intestinal parasites may tend to reduce the well-being of the mother. So, too, the demands of multiple childbearing are an added factor. All in all, it is clear enough that the infant who is a potential subject to develop kwashiorkor comes from maternal stock which is as poor nutritionally as one could expect to find.

That the infant is poorly prepared is shown by analysis of his food, i e., mother's milk, and the content of certain nutrients in his own organism at birth. Analyses of human milk for certain vitamins, minerals and proteins have shown decreases. So, too, analyses of the serum levels of certain of these materials in the newborn have indicated reductions from the accepted standards for well-nourished infants at birth

Be this as it may, no clinical disease is particularly evident while the infant is nursing, even if he is put to the breast of a foster mother. The nursing period, which varies in different areas of the world, has been surveyed by Jelliffe.[1225] During this time the infant is exposed to any number of insect and water-borne diseases, particularly malaria in certain areas The role of such infections has not yet been closely delineated nor have the protective effects of antibody derived from the mother *in utero* or in the maternal milk been assayed. It is this preweaning, incipient kwashiorkor period of which we have least information, either from clinical or pathological studies, since most observations until recently have been restricted to severely ill infants and children requiring hospitalization.

At any rate, weaning, which may be at six months or as late as three years, brings about ration consisting of a porridge of maize or some other grain From now on, though growth may have been proceeding at a slow rate, the growth curve may flatten out or may even drop in comparison with what should be expected. In such children one may begin to find *periportal fatty infiltration of the liver at autopsy if they die of some infectious or other disease*, or edema of the dorsal portion of the feet may be detected; or anorexia and listlessness may become apparent. Examination of the blood constituents before florid kwashiorkor manifests itself

may reveal anemia and decrease in concentration of albumin, cholesterol, certain vitamins and minerals Skin lesions may also develop

The stage appears to be set for clinical kwashiorkor, which so frequently is ushered in by diarrhea The infant overnight becomes severely ill Edema increases, diarrhea is excessive, the child becomes more apathetic and skin changes become more severe This is the florid clinical picture which Cicely Williams described for us at the beginning of this section.

Various forms of treatment have been tried Most successful have been milk supplements Vitamins and minerals are not effective Protein appears to be the decisive factor and recently Brock [1244] has administered crystalline amino acids with success

Several questions remain to be asked and discussed. First, what is the relation of kwashiorkor to marasmus? By the latter term one means starvation in infancy. The group at INCAP in Guatemala City has commented on this [1221] and have schematically represented the two syndromes as forming the base of a pyramid with the various manifestations of each varying in degree so as to make up the full-blown syndrome of one or the other This is a most satisfactory way to regard the situation clinically and empha-

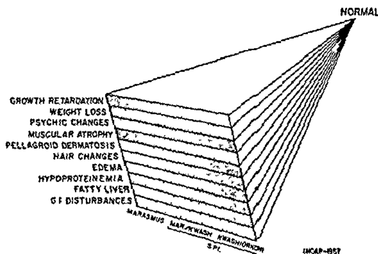


FIGURE 133 THE PYRAMID OF MALNUTRITION.

Developed by the professional staff of the Institute of Nutrition of Central America and Panama (INCAP), with the assistance of Dr. J M. Bengoa, Inter-Regional Advisor in Nutrition, WHO.

sizes, too, the importance of caloric intake, growth, et cetera, on the two diseases.

Another question which needs comment is, what is the relation of kwashiorkor to pellagra? Is kwashiorkor the infantile form of pellagra? The answer to this is not clear. Many of the manifestations of the two syndromes are the same Both are related to similar dietaries. The lesions are alike, save that the dermatitis in children does not appear to be affected by sunlight It is of interest that so little attention appears to have been given this possible relationship in past years. Part of this would appear to be due to the general loss of interest in pellagra. This is evinced by the decrease in publications dealing with the syndrome. The situation for kwashiorkor is quite the reverse It is significant that at a recent conference held in Jamaica on the subject of protein malnutrition[1226] pellagra was hardly mentioned.

Finally, it is pertinent to ask, since the history of deficiency disease teaches us that much is to be gained from studies of human-like syndromes in experimental animals, what has been done to reproduce a kwashiorkor-like disease in animals? One might suppose that the most obvious way to attack this problem would be to feed animals diets comparable to those taken by infants who develop kwashiorkor. This is just what was done during the quarter century (1917-42) when blacktongue in dogs was being studied. Kwashiorkor, however, had hardly appeared on the scene at the time when interest in pellagra and blacktongue began to wane.

The problem has been pointedly studied in rats. Fatty liver followed by cirrhosis has been observed in rats fed a maize meal diet, supplemented with fermented milk.[1245] Periportal fat infiltration has been observed in rats fed corn meal, supplemented with salts and vitamins[1246] We have studied rats which were fed exclusively on corn meal.[1251] Nothing resembling a kwashiorkor-like syndrome can be produced in this species, however. One finds retardation of growth, alopecia, hyperplastic goiter, changes in hypophysis, and rickets. On the other hand, a syndrome rather similar to kwashiorkor has been observed in monkeys.[1247] This is characterized by growth failure, apathy, fatty liver, and hypoproteinemia due to hypoalbuminemia. Much more needs to be done to delineate the pathogenesis of this syndrome

NUTRITIONAL LIVER DISEASE IN MAN

During recent years clinical and pathologic studies of liver disease in man have focused attention on the role of nutrition in the causation of several syndromes which are primarily hepatic in origin. As might be expected, experiments designed to produce necrosis and cirrhosis in experimental animals which were discussed on pages 98 and 255 have simulated studies in the human. Naturally, too, since certain obvious deficiency disease syn-

dromes, such as pellagra and kwashiorkor, are featured, in part, by involvement of the liver, investigations dealing with the relation of diet to other types of hepatic disease were clearly indicated. During the past fifteen years great strides have been made in our understanding and recognition of certain nutritional disease syndromes which are characterized by severe hepatic involvement, though as will shortly become apparent, the precise pathogenesis of each in the human subject is still far from clear [1249, 1250]

As it occurs in the human, nutritional liver disease can be conveniently separated into three fairly distinctive syndromes (1) *Simple fatty liver*, (2) *Fatty liver with insufficiency*, and (3) *Cirrhosis* With few exceptions, in all of these syndromes the primary defect, which may be encountered on biopsy or at autopsy, is a fatty liver The most common underlying factor leading to dietary insufficiency is alcoholism If one biopsies the liver in a group of alcoholics, 70 per cent are found to have fatty infiltration [1252] Fatty liver and alcohol ingestion go hand in hand in each of the three syndromes which we shall discuss

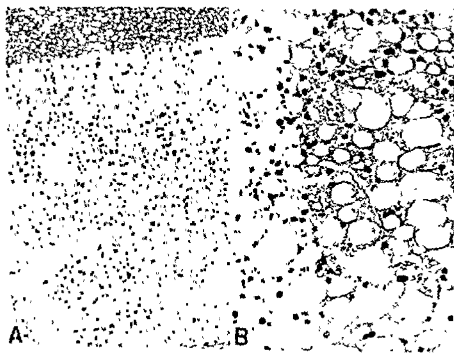


FIGURE 134 NUTRITIONAL LIVER DISEASE

Biopsy Human Fatty infiltration in an alcoholic who had an enlarged liver at the time of biopsy Dietotherapy led to reduction in size of liver.

The first entity, simple fatty liver, manifests itself in a patient, usually a male, who has an enlarged liver, which biopsy reveals to be filled with lipid. Relatively little else may be found on clinical examination. A history of excessive alcohol consumption may usually be elicited. The individual appears well-nourished and may have few, if any complaints. The spleen is not enlarged. Chemical tests of liver function are not abnormal unless the fatty change has been present for some time; then there may be bromsulphalein retention. So, too, some degree of hypoalbuminemia may be present, usually not enough to lead to edema.

Ordinarily such a patient does not come to autopsy unless an accident has occurred, or some infection brings him to the hospital. If one is able to examine such a liver under the microscope, he will find varying degrees of fatty infiltration which bear little relation to the liver lobule, though sometimes there may be more about the central vein than elsewhere So many other factors such as anemia, cardiac failure, et cetera, affect the lobular distribution of lipid usually one hesitates to assign much importance to its variations.

The pathogenesis of this change is, as noted above, to be ascribed to the notoriously poor diet of the alcoholic. If an individual is consuming one fifth of whiskey or gin a day, he is perfectly able to maintain his caloric requirements, particularly if he is not working. Since his diet usually lacks protein and sources of vitamins, particularly of the B-group, deficiencies of these materials lead to the fatty infiltration. If diet therapy is instituted, a prompt decrease in the size of the liver occurs; biopsy reveals a disappearance of lipid. Any hepatic tests that were abnormal will revert to the normal range.

The second syndrome, fatty liver with insufficiency, is a much more dramatic and serious one.[1259] The basic defect in the liver is the same as in the first syndrome; however, as chemical studies will show, things have progressed much further. A prominent feature of this syndrome is its sudden onset. An individual who may or may not have been known to have fatty liver disease suddenly becomes jaundiced. Frequently the precipitating factor is an infection Sometimes acute nutritional deficiency associated with an alcoholic spree may initiate the syndrome.

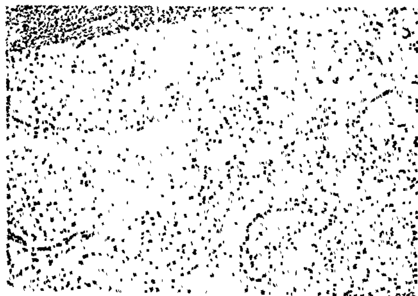
Clinically, such a patient has an enlarged, sometimes tender liver. He is jaundiced. Serum bilirubin is elevated; other liver function tests: cephalin floculation, thymol turbidity, bromsulphalein retention, prothrombin time, serum alkaline phosphatase, are all abnormal. Hypoproteinemia may be marked and is accompanied by edema. Hemorrhage in various areas may be prominent. Death may occur in coma, seemingly unaffected by therapy.

At autopsy, the liver is large and greasy. It turns green in formalin and

may even float in the fixative. Under the microscope, fatty change is extreme. This is corroborated by chemical analysis [1253] Usually an increased amount of connective tissue is found about the central veins, but relatively little other increase is seen in other areas Bile plugs are frequently found in small ducts Focal necroses are usually present.

The rapid progress of this syndrome is related to acute liver failure. The presence of necroses indicates that hepatic damage has gone further than simple fatty change. As noted above, the commonest precipitating factor seems to be infection, though sudden and increasingly severe dietary deficiency associated with alcoholism may be equally important.

The third syndrome is cirrhosis This term, which Laennec introduced to describe the color of the liver, has now come to mean scarring Hence, it refers to any condition in which liver cells are destroyed and replaced by connective tissue fibers The syndrome of *Laennec's portal cirrhosis* presents definite clinical and pathologic pictures [1255, 1256] We shall not go into all of these aspects in any detail here since the disease is so well-known. Certain considerations are important, however As in the two preceding syndromes, diet and alcohol appear to form the foundation for the development of the disease Hence, *the early stages are characterized by fatty liver* It is therefore not surprising that each of the syndromes already



DISEASE

Port ... liver exhibiting coarse scarring From male with histo... ...ing of hematemesis

mentioned may progress slowly or rapidly into the syndrome of full-blown cirrhosis.

The major clinical signs of the disease are: ascites, peripheral edema; jaundice, though not always; enlargement of the liver and spleen; telangiectases, and hematemesis. Less prominent signs are dermatitis, gynecomastia, hypotrichosis, spider nevi, and palmar erythema. Laboratory tests reveal: anemia, sometimes macrocytic in character; hypoproteinemia with hypoalbuminemia and reduction in fibrinogen; increased non-protein nitrogen of serum; and increased serum bilirubin. A number of other routine liver function tests indicate hepatic dysfunction.

At autopsy the liver characteristically shows diffuse coarse or fine scarring or varying degrees of both. Microscopic examination reveals that the tissue is broken up by coarse or fine bundles of connective tissue in which there are variable numbers of inflammatory cells and proliferating bile ducts. The liver cells themselves may show necroses, fatty infiltration and other evidence of damage. Many nodules of hyperplastic liver cells may be found The spleen is usually enlarged. Other findings, such as ascites, varices, et cetera, are related to the effects of circulatory obstruction in the liver.

In contrast to the second syndrome, death in this third type of liver disease does not ordinarily result from hepatic insufficiency, but usually occurs from complications arising from obstruction to the flow of blood through the liver.

The foundation for these three situations is fatty infiltration of the liver. In the first type the beneficial effects of diet are well-recognized. A similar response is found when fatty liver with insufficiency is present and has not progressed far enough. This is a difficult point to determine with certainty. Sometimes the patient who does not appear too severely ill goes on to exitus despite therapy, while individuals who clinically appear more ill recover. It may be that the determining factor is not fatty infiltration but actual necrosis of the hepatic cells. It is likely that death of liver cells may be a link between less severe types of the second syndrome and cirrhosis. Much has been written concerning the pathogenesis of cirrhosis, which in many instances clearly appears to have a nutritional background.[1254, 1257] Some are content to liken the disease in man to what is seen in the experimental animal Recent studies of experimental cirrhosis in rats in which antibiotics have been administered would appear to make the transition of fatty liver to cirrhosis in the rat more complicated than was originally believed. Others feel that the pathogenesis of cirrhosis is more complex and the progression is influenced by infections in particular but also by anemia, cardiac failure and other adverse conditions. An important consideration is the inability of the cells to regenerate on the one hand which leads to their replacement

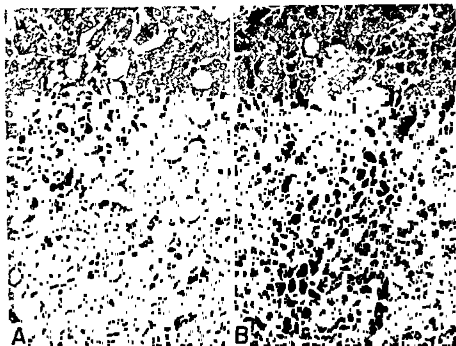


FIGURE 136 NUTRITIONAL LIVER DISEASE

Human *A* and *B* (x 250) Early scarring, fatty infiltration and hydropic change in liver shown in Figure 135.

by connective tissue and the presence of hyperplastic nodules which compress neighboring areas and help to produce the circulatory disturbances which are such a dramatic part of cirrhosis [1258] The consequences of these circulatory disturbances within the liver are more foci of necrosis which make matters even worse Nutritional liver disease of which cirrhosis is the most important type exemplifies all of the complexities of naturally occurring disease and should teach us that it is unwise to transfer relatively simple entities which may be produced in the experimental animal to such a complex organism as man in his natural environment.

THE HYPOGLYCEMIC SYNDROME

Elsewhere (page 117), the dispensability of preformed carbohydrate in the diet of the rat has been noted Whether a similar situation might apply to man is not clear and deserves further study. For instance, von Chwalebowski[1260] reared a child from birth on a diet said to be "practically carbohydrate-free" At ten months of age, when the infant evinced ketosis, the experiment was stopped.

Both protein and lipid in the diet give rise to carbohydrate which is transformed into glycogen in the liver in order to maintain a constant serum glucose level The cells of certain tissues, particularly neurons, are almost exclusively dependent on glucose for their energy requirements. Hence the level of glucose in the circulating blood is of great importance to these structures. A drop in glucose concentration naturally leads to deficiency disease as far as such tissues are concerned How may the levels of serum glucose be depressed in the human and so bring about the hypoglycemic syndrome?

As in the experimental animal, naturally occurring hypoglycemia results from disease in two main areas the liver and certain endocrine organs. A simplified classification is presented in Table XI

TABLE XI

SOME ETIOLOGIC FACTORS PRODUCING THE HYPOGLYCEMIC SYNDROME

I *Disease of the Liver*
- (1) Massive necrosis (acute yellow atrophy)
- (2) Infectious hepatitis
- (3) Cholangiolitis
- (4) Diffuse fatty infiltration
- (5) Glycogen storage disease
- (6) Diffuse replacement by tumor cells

II *Endocrine Disease*
- (1) Hyperinsulinism
  - (a) Excess administration of insulin
  - (b) Insuloma, benign or malignant
  - (c) Hyperplasia, islet cells
- (2) Pituitary hypofunction
- (3) Adrenal cortical hypofunction

III *Miscellaneous*
- (1) Starvation
- (2) Severe, continuous muscular work
- (3) Pregnancy in ruminants

Since the liver is the primary site of glycogen storage and release, this type will be mentioned first although hypoglycemia resulting from hepatic disease is not common. When such does occur it can be assumed that involvement of the liver is extreme; hence, other more serious effects of liver dysfunction may mask the manifestations of hypoglycemia.

It would not appear necessary to discuss each type of liver disease in turn Those listed in Table XI are now well-recognized.[1263 1264]

The first disturbance in the endocrine group (Table XI) stems from hyperinsulinism. The symptoms of excess insulin administration, which were described shortly after the discovery of the hormone in 1924,[1261, 1262] will be mentioned below. The most interesting group of cases is that in which one or more insulin-forming tumors of the islets of Langerhans are present. Such tumors are not common, up to 1919, 258 cases had been reported in the literature [1265]

Patients with hypopituitarism, whether due to circulatory disturbances, tumor involvement, or "idiopathic atrophy," may evidence hypoglycemia. A complicating factor in such cases may be the presence of hypothyroidism All patients in this group are abnormally susceptible to insulin.

Finally, one may encounter the hypoglycemia syndrome when there is disease of the adrenal cortex due to destruction by some inflammatory process, replacement by tumor, or idiopathic atrophy. The degree of hypoglycemia would appear to be related to the extent of destruction of the adrenal cortices.

The miscellaneous group listed in Table XI could be expanded, particularly to include the somewhat poorly-understood and overly-diagnosed cases of functional hypoglycemia. It is recommended that such a diagnosis should be made with great care.[1250]

What are the signs and symptoms of the hypoglycemia syndrome? They can best be described by drawing on the group of "pure" hypoglycemics, that is individuals having hyperinsulinism resulting from adenomas of the islets of Langerhans.[1265] The symptoms can be separated as to whether they are produced by excess insulin, by excess epinephrine or by hypocorticism The primary effects of hyperinsulinism, i e., hypoglycemia, are mainly neurological and are, in order of decreasing frequency, as follows. loss of consciousness, confusional state, convulsions, loss of continence, amnesia, positive Babinski, transient hemiplegia, and paresthesias.

Since a low blood sugar level stimulates the release of epinephrine, some of the effects of an excess of this hormone may be prominent; these are sweating, tremor, pallor, coldness, palpitation and procordial oppression. Finally, the effects of hypoadrenalism may be combined with those of hypoglycemia; these are weakness, fatigue, light-headedness and visual disturbances.

Ordinarily, these manifestations of hypoglycemia make themselves known so that diagnosis is not too difficult; hence, the degree and duration of the low blood sugar is not overly prolonged. If the hypoglycemic episode is severe and of some duration, actual damage to neurons may be found at autopsy (page 118). Such changes resemble those which may be seen following severe hypoxia and other non-specific metabolic defects. As might be expected, the earliest morphological change is an alteration in the Nissl substance of neurons, this is reversible. As time goes on, however, the neuronal damage becomes irreversible and necrosis occurs. Such dead neurons are taken up by phagocytes. There is some glial reaction. Petechial hemorrhages may be prominent in the brains of individuals dying as a result of acute hypoglycemia.

Hypoglycemia may be seen in farm animals,[1491, 1492] particularly ewes and cows during the last months of pregnancy. Hence, the term "pregnancy toxemia" has been given to this disturbance. The signs are those of hypoglycemia. It has been assumed that they are due to a hypoglycemic encephalopathy. As might be expected, ketosis is also a prominent part of this syndrome.

XEROPHTHALMIA and OTHER MANIFESTATIONS OF HYPOVITAMINOSIS A

One might suspect that the alterations, which have already been described in the experimental animal deprived of vitamin A, might also be found in the human. Such is the case. However, the multiple lesions which may result from vitamin A deficiency, together with their effects, are usually overshadowed by the symptoms and signs of the disease process which is responsible for the deficient state. Hence, with a single exception, a clear-cut syndrome of hypovitaminosis A cannot be described. We shall comment below on the alterations which may be encountered in various epithelial tissues. So, too, the questionable position of the skin lesions, i.e., the papulofollicular eruption or phrynoderma, as part of the vitamin A deficiency state will be discussed. Here, however, it is necessary to describe what to our mind is the only precise clinical and pathologic syndrome which can be ascribed to vitamin A deficiency. This is xerophthalmia and its associated ocular changes.

Historically, xerophthalmia has been recognized for centuries. But until 100 years ago the disease did not gain such prominence that its possible relation to improper diet could begin to be appreciated and anatomical alterations in the eye could be closely associated with night blindness or hemeralopia. This condition occurs in various diseases and manifests itself by an inability to see in dim light. The coincident occurrence of hemeralopia and a lesion of the conjunctiva was communicated to the Imperial Academy of Medicine in Paris on April 28, 1862, by Pierre Bitot.[1271] The lesion, which he had observed among the children in a Bordeaux hospital, was described as follows:

"It is triangular, its tip external, its base adjacent to the cornea is a little concave. In some cases it is circular or oval, in others, simply linear. Most often the particles which compose it are agglomerated in a way to produce a punctate, granular surface, at other times these particles are arranged in series of wavy parallel lines, which give the lesion the appearance of an undulating or rippled surface." Going on, B [illegible]

[illegible] epithelium."

During the ensuing years, others described this association of hemera-



FIGURE 137. XEROPHTHALMIA.

Conjunctiva, human. *A* Area of conjunctiva which has "whitish soapy" appearance conforming with modern day description of "Bitot spot." (Courtesy of Dr H. A P. C. Domen.) *B* (x200). Microscopic section of hyperkeratotic lesion which might be expected to conform to pathologic appearance of "Bitot spot," (A.F.I P. 308895). There was no reason to suspect vitamin A deficiency in this instance, however

lopia and xerosis conjunctivae. Such cases were seen in individuals with malnutrition resulting from periods of fasting or with faulty dietary intake in various areas By the turn of the century the causal relation of faulty nutrition to the eye changes seemed clear enough for Mori [1272] to suggest that the ocular lesions which he had observed in a large number of Japanese children were due to a lack of fat in the diet. He was clearly aware of the virtual absence of fat from the children's daily foodstuffs, particularly prominent was the low-fat content of rice. Moreover, he indicated that the Japanese failed to receive any milk after weaning.

In the next decade the prevalence of xerosis and keratomalacia in poorly

nourished children was commented upon time and time again The syndrome of Mehlnahrschaden, which had come into prominence at this time, was accompanied by keratomalacia.

By 1914, the stage was set for the pathogenesis of xerosis to be clarified A fat-soluble active material (vitamin A) which is present in certain foodstuffs had been shown to enhance the growth of rats on a semi-purified diet. Both Osborne and Mendel and McCollum and Davies, who had discovered vitamin A, had noted changes in the eyes of the deficient rats. Such lesions were cured by feeding the new fat-soluble factor. In 1915, Goldschmidt [1273] studied the ocular tissues of rats which he had placed on a semi-purified diet without the protective material He found the lesions of human xerosis.

The final proof came during the ensuing few years when Bloch [1274] and Blegvad [1275] correlated the prevalence of xerophthalmia and keratomalacia in Denmark with the availability of vitamin A—containing foodstuffs. These studies, which are of extraordinary interest, dealt with cases of xerophthalmia which had resulted from a fall in vitamin A consumption, then a rise, followed by another reduction, all of which clearly showed the relation of vitamin A to ocular disease During the same period, tests for hemeralopia were beginning to be utilized in an attempt to detect vitamin A deficiency.[1276]

What are the clinical and pathologic manifestations of these ocular changes? The initial disturbance is night blindness, a defect, physiologic in origin, which renders an individual unable to see in dim light The situation was described in China by Pillat [1277, 1278] as follows. "in mild cases the patients are unable to read or do any fine work in the evenings, in severe cases, patients lose their orientation on the street after sunset and cannot find their way home." No eyeground changes are present. The disturbance is usually the first manifestation of vitamin A deficiency, hence no other signs or symptoms need be present

Xerosis follows hemeralopia as the second stage of vitamin A deficiency This conjunctival change may appear in several forms. (1) As *Bitot's spots*, which are foam-like and intensely white, and are found on the temporal side, extending from the limbus to the lens angle. Tear fluid does not adhere to these spots. (2) Larger and irregular areas, which progress to excessive wrinkling of the bulbar conjunctiva which loses its lustre. (3) Corneal involvement, as manifested by loss of lustre, reduced sensibility and the presence of desquamated debris This may be followed by (4) the end stage, keratomalacia This leads to perforation with resulting herniation of various internal structures. Other characteristic changes which have been described are pigmentation of the conjunctiva, meibomitis, blepharitis, comedones, and edema of the lids

Certain disturbances accompany these alterations: photophobia, burning and dryness of the eyes.

The pathologic alterations on the human eye are similar to those already described in the experimental animal (page 128). Such include hyperkeratinization of the corneal epithelium, cellular infiltration, and vascular growth into the substantia propria. Similar hyperkeratotic changes are found in the conjunctiva and the ducts of the paraocular glands. The latter may then become atrophic.

As already noted, these ocular changes comprise the most specific syndrome of vitamin A deficiency in the human. Certain other tissues may be found to be affected at autopsy. This is particularly true in several series of cases which have been reported from Boston [1279] and from China.[1280] Before discussing these alterations it might be well to mention briefly how the organism comes to be deprived of vitamin A. Three main factors appear to operate In the first place dietary intake may be reduced. This is true of the deficient state associated with *Mehlnahrschaden* [1514] and the "epidemics" which have been described, such as the well-known one in Denmark.[1274, 1275] Secondly, any disturbance of the gastrointestinal tract and its associated structures, particularly pancreas and biliary system, may interfere with the absorption of vitamin A.[1284] Such disturbances include diarrhea, celiac disease, sprue and steatorrhea, as well as cystic fibrosis of the pancreas and biliary atresia. Finally, excessive needs, such as those during active growth or as a result of heightened metabolism, may play a role.

At autopsy children may exhibit typical keratinizing metaplasia in the kidney pelves, the bladder, the lining of the nasal sinuses, the respiratory tract and in other tissues similar to those affected in experimental animals. Of particular interest are the pulmonary lesions which consist of bronchitis, bronchiolitis and lobular pneumonia, all of which may be extreme. In the absence of normal ciliated epithelium, bacteria are not disposed of in the usual fashion and are able to grow, invade the bronchial walls, and produce inflammation of the surrounding structures.

Changes similar to those observed in the enamel organ of the rat have been described in this structure of an infant exhibiting other manifestations of vitamin A deficiency, but since the general incidence of vitamin A deprivation in children is so low, it is unlikely that a deficiency of this nutrient is ever a common cause of enamel hypoplasia in the human [1281]

At autopsy, morphological evidence of vitamin A deficiency in the adult is much more uncommon than in children. In fact, aside from the clinical manifestations referred to above, instances of clear-cut examples of keratinizing metaplasia in the adult are virtually non-existent except for the occurrence of xerosis in the eye It should be pointed out that several years ago, based upon observations in animals and little else, vitamin A de-

ficiency was introduced as a prominent cause of urinary calculi in man. Studies by Jewett *et al.*,[1282] who utilized the dark adaptation technique and whose material was examined by the present writer, would seem to show conclusively that vitamin A deficiency is a rare cause of urinary calculi in man, in this country at least.

Finally, mention must be made of the skin lesions which have been assumed by many to be specific for vitamin A deficiency. Frazier and Hu[496] in 1931 called attention to dryness and roughness of the skin in individuals who might have been deficient in vitamin A. Particularly prominent were spinous papules at the sites of hair follicles. This change was seen first over the anterior and lateral aspects of the thighs and posterolateral surfaces of the upper forearms. Spread took place to the exterior surfaces and over the shoulders and trunk, back and buttocks. The skin might have a dull slate color. The follicular papules measured up to 5 mm. in diameter, each had an hyperkeratotic plug in the apex. Frazier and Hu[496] were aware that similar changes might occur in scurvy. As time went on the relation of the change to vitamin A deficiency was taken for granted. The term phrynoderma (toad skin) was coined.

However, others doubted the relation of the dermal changes to vitamin A deficiency. For instance, no correlation could be found with incidence of eye change and skin lesions. Moreover, the experimental study carried out by the British Medical Research Council[524] failed to reveal any specific follicular changes. Hence, at the present time this aspect of vitamin A deficiency must be looked upon with some question. The possibility that phrynoderma may be related to some other deficient state, such as that produced by a lack of essential fatty acids, must be considered; this has been discussed recently.[445]

The exact incidence of hypovitaminosis A in the world today is not precisely known. Studies in China a decade and a half ago would indicate that vitamin A deficiency was widely prevalent.[1283] It has been seen more recently in association with kwashiorkor.[1285] A critical appraisal of vitamin A deficiency to naturally occurring eye lesions and the effects of other deficiency states thereto is certainly indicated.[1286] Much now would appear to hinge on the definition of vitamin A deficiency, on the meaning of Bitot's spots,[1092] on the significance of follicular hyperkeratosis, and on the possible value of tests for dark adaptation.

RICKETS AND OSTEOMALACIA

Rickets may be defined as a disease of the growing skeleton which is characterized by a decreased concentration of hydroxyapatite in the organic matrices of cartilage and bone. Osteomalacia is rickets in the adult and, since cartilage growth has ceased, is to be defined as a skeletal disease in adults which is characterized by a decreased content of hydroxyapatite in bone matrix (osteoid) Rickets can be diagnosed on chemical grounds or, if one can demonstrate areas of matrix devoid of mineral in cartilage or bone under the microscope, on anatomical grounds The normal inorganic content (ash) of dried, fat and marrow-free bone is about 60 to 65 per cent A level of ash content at which rickets or osteomalacia might be said to exist has never been definitely stipulated Values below 50 per cent might be taken to indicate that rickets or osteomalacia is present

In the discussion to follow we shall take up briefly the historical development of our knowledge of rickets and osteomalacia, then discuss in some detail the pathogenesis of the two diseases, and finally indicate the pathological, clinical, and roentgenological changes which may be encountered

From the time of Glisson[1183] in the seventeenth century, the skeletal deformities of rickets have attracted great interest However, the chemical basis for the softening of the bones was not shown clearly until almost the middle of the nineteenth century, when Marchand[1290] presented chemical analyses of the skeletal tissues from rachitic children, such studies revealed a decrease in the total ash content, which in large part was due to a reduction in concentrations of calcium and phosphorus. Shortly thereafter the results of other analyses appeared So, too, the first chemical determinations were reported on the bones from cases of osteomalacia.[1291] These demonstrated reductions in calcium and phosphorus concentrations. While this chemical basis for an understanding of rickets was being developed, histological studies of the deformed bones were also being carried out. The first microscopic report is that of Rufz in 1834[1292] A clear understanding of the disease was not provided, however, until 1858[1293] when H Muller clearly described osteoid in the shaft, defective calcification of the cartilage, and the healing phenomenon which now bears his name. The pathologic anatomy of rickets was so thoroughly elucidated by Pommer in 1885[527] that very little has remained to be added In 1909, Schmorl[1294] presented his classic studies on the incidence of rickets in Dresden during the years 1902-07. During this period Schabad[1295] pointed out that losses of calcium and phosphorus may be demonstrated in children with rickets A little over 10 years later came the end of the story. Howland and Kramer[1296]

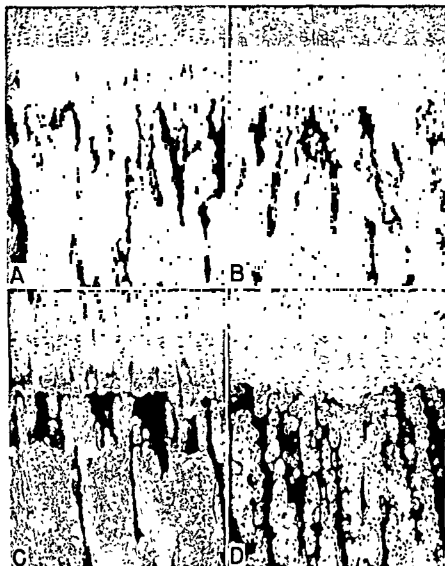


FIGURE 138 RICKETS

Cartilage-shaft junctions, human Various patterns of defective calcification in cartilage *A* H L H, 947 (x 75), *B* H L H, 1174 (x 65), *C* H L H, 947 (x 75), *D* H L H, 1015 (x 100)

TABLE XII

THE PATHOGENESIS OF RICKETS AND OSTEOMALACIA

I *Disturbance in Balance of Matrix Production and Deposition of Hydroxyapatite*
- (1) Rapid matrix formation, particularly in the premature infant
- (2) Healing fractures
- (3) Healing scurvy
- (4) Healing bone following removal of parathyroid tumor

II *Disturbance in Intestinal Absorption of Calcium and/or Phosphorus*
- (1) Calcium
  - (a) Dietary lack
  - (b) Change in pH of intestinal contents
  - (c) Formation of insoluble complexes: oxalate, phytin
  - (d) Protein content of diet
  - (e) Steatorrhea, sprue
  - (f) Vitamin D lack
    - (aa) Dietary
    - (bb) Steatorrhea
    - (cc) Absence of bile
    - (dd) Absence of pancreatic juice
    - (ee) Impaired formation in skin
- (2) Phosphorus
  - (a) Dietary lack
  - (b) Change in pH of intestinal contents
  - (c) Steatorrhea
  - (d) Formation of insoluble complexes

III *Excess Excretion of Calcium and/or Phosphorus*
- (1) Renal Disease
  - (a) Glomerulo-tubular
  - (b) Tubular (frequently hereditary)
    - (aa) Phosphate diabetes
    - (bb) Phosphate diabetes with glucosuria
    - (cc) Fanconi syndrome (phosphaturia, glucosuria, amino aciduria)
    - (dd) Renal tubular acidosis
  - (c) Idiopathic hypercalcuria
- (2) Pregnancy, lactation

pointed out the importance of the product of the serum calcium and phosphorus levels in relation to the pathogenesis of rickets in children, and Park and Howland[1297] described the curative effects of vitamin D by x-ray study of the skeleton. The production of rickets in experimental animals and the interrelations of calcium, phosphorus and vitamin D are discussed on page 141. Such are the highlights of the story of rickets. From the number of contributions which were made and continue to appear today the problem is far more complex than this brief historical review might indicate.

From what has already been said concerning the definition of rickets

and osteomalacia and of the studies of Howland and Kramer[1296] which were just so briefly alluded to, it should be clear that any factors which may lower the concentrations or availability of serum calcium and/or phosphorus may effectively prevent the deposition of hydroxyapatite crystals in the organic matrices of cartilage and bone What factors are able to do this and how may they operate? A summary of the pathogenesis of naturally occurring rickets and osteomalacia is presented in Table XII The conditions which operate are somewhat similar to those which have been shown to lead to experimental rickets (Table VII, page 000). It will be noted *that four main possibilities exist.* decreased *intestinal absorption of calcium,* impairment of intestinal absorption of phosphorus, increased urinary excretion of calcium and increased renal loss of phosphorus In addition, though optimal humoral concentrations of calcium and phosphorus may be present, bone and/or cartilage matrix production may be excessive, the matrix may be immature, or some defect may be present to block the calcification mechanism

Ordinarily when the organic portion of bone, i e, osteoid, is formed, hydroxyapatite crystals deposit in it immediately, since the balance between matrix production and the deposition of inorganic elements is such a delicate *one Under certain circumstances matrix production may be excessive, then* even though the concentrations of calcium and phosphorus, as mirrored by serum levels, are seemingly optimal, deposition of inorganic salts does not keep pace Rickets or osteomalacia is then to be found For instance, the rapidly growing premature baby exhibits zones of osteoid which may be in excess of what one would wish to call "physiological" in the full-term infant[1298] So, too, the callus of healing fractures shows similar areas of uncalcified, newly-formed matrix[1299] In this situation the matrix itself may not be "mature" enough for calcification to take place. A similar situation is seen in healing scurvy where incipient rickets may be brought out as a result of excessive osteoid formation[1327] The bones of individuals from whom a parathyroid tumor has recently been removed may show an overabundance of new bone matrix.[152] Here, of course, the serum levels may reflect not entirely optimal interstitial fluid concentrations of calcium and phosphorus for lime salt deposition to take place The last example to deserve mention is osteopetrosis or marble bone disease. Here, normal *bone destruction is impaired* or *completely lacking* Hence, large amounts of ordinarily available calcium and phosphorus are "locked up" in the skeleton and are not available for their usual normal redistribution In our experience marble bone disease is usually accompanied by rickets[1441]

A large number of factors may affect the absorption of calcium and/or phosphorus from the intestinal tract. With respect to calcium, dietary restriction is of importance,[1316, 1449] though in man uncomplicated calcium de-

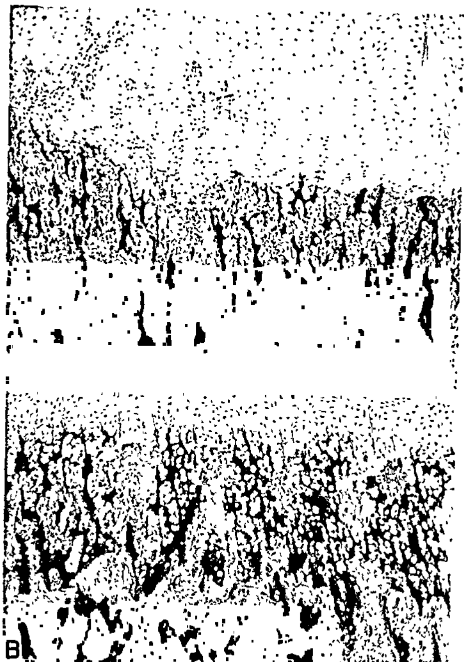


FIGURE 139 RICKETS.

Costochondral junctions, human *A*. H L H, 1363 (x 75), *B* H L H, 981 (x 75). Defects in calcification of cartilage.

ficiency must be most uncommon this is not true in animals in which naturally occurring osteomalacia has been described [1442 1443 1444 1445] Calcium absorption is affected by the pH of the intestinal contents, acidity facilitates, while alkalinity inhibits calcium uptake [1300] Any number of acidic radicles may form insoluble complexes with calcium, such include, oxalate (spinach)[1301] and, particularly, phytate [1302] The latter is of importance with respect to cereal grains in which it is found in large amounts. The protein content of the diet affects the absorption of calcium, a high intake promotes an increase and conversely low intakes of protein diminish calcium absorption [1303] Large losses of calcium from the intestine may result from diarrhea, particularly in the syndromes associated with steatorrhea [1304 1305 1306] These disease states may be present in the absence of any clinical evidence of deranged intestinal function

The dietary intake of vitamin D is of the utmost importance to the growing child as far as calcium uptake is concerned [1310] The absorption of vitamin D may be impaired by a variety of circumstances Syndromes such as sprue and steatorrhea, which interfere with the absorption of lipids,



FIGURE 141. RICKETS

Costochondral junction, human A seven months old child dying of unexplained fever and diarrhea H and E. (x 15).

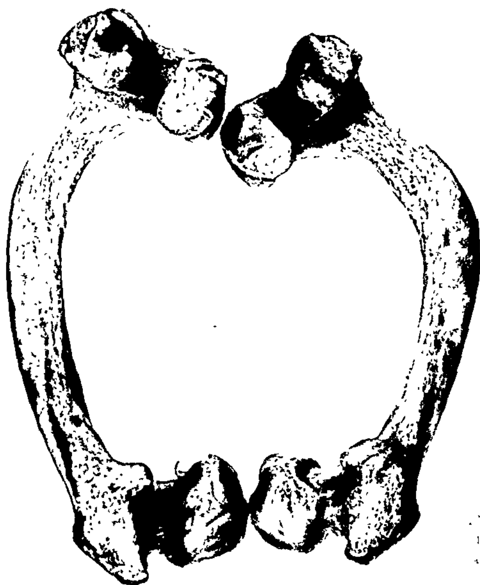


FIGURE 140. RICKETS

Skeleton, human Gross deformities of femurs produced by rickets

usually explained as follows.[152] Tubular disease leads to acidosis which is followed by hypercalcuria and hypocalcemia The latter gives rise to parathyroid hyperplasia, which is ineffective as far as phosphate excretion is concerned. Hypocalcemia and hyperphosphatemia are the rule, the latter is due to tubular dysfunction Even though the product of the serum concentrations of calcium and phosphorus is high, excess osteoid is frequently seen. The reason for this is not entirely clear. The excess destruction (osteitis fibrosa), which is observed, is usually ascribed to parathyroid stimulation by depressed serum calcium levels.

In recent years an increasing number of isolated renal tubular defects have come to be recognized. These are associated with an inability to resorb certain metabolites, such as water, phosphate, glucose, cystine and other amino acids, calcium, bicarbonate, potassium and sodium, from the glomerular filtrate.[1311] Certain of these defects may lead to rickets or osteomalacia.



FIGURE 143 RICKETS

Shaft Osteoid borders about trabeculae from section shown in Figure 142 H and E (x 60).

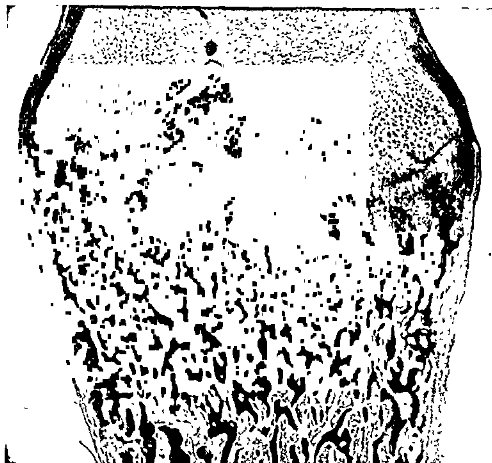


FIGURE 142 RICKETS

Costochondral junction Costochondral junction from a seven months old male dying acutely of lobar pneumonia. H. and E. (x 15).

depress the uptake of vitamin D. Since bile and pancreatic secretions are also of importance in this respect, rickets is seen in children with congenital atresia of the bile ducts [1307] and in cystic fibrosis of the pancreas.[1323] The effect of sunlight is important, too [1308]

Of the disturbances in excretory function which may affect the metabolism of calcium and/or phosphorus, those which are associated with the three most prevalent forms of chronic nephritis: vascular, glomerular and pyelonephritis, are the most common.[1446 1447] Two changes in the bones are prominent: excessive destructive activity (osteitis fibrosa) and the presence of excess osteoid (rickets or osteomalacia). These alterations are

prefer the term, phosphate diabetes, as do others, and look at the disease as a specific tubular defect *unrelated* to vitamin D "resistance"

Several other syndromes closely allied to the above are seen, in all of which rickets or osteomalacia is present Phosphaturia may be accompanied by glucosuria. Other cases may show amino aciduria with or without loss of potassium and, in addition, defective acidification of the urine, these fall into the designation of "Fanconi Syndrome."

The renal acidosis syndrome[1314] must be looked upon as another primary disturbance of the renal tubule. This is seen in infants, adolescents or adults and may have a familial incidence The primary disturbance appears to be an inability to produce a urine of normal acidity The urine, which is of increased volume, is alkaline with fixed specific gravity Acid metabolites are excreted with fixed base so that a constant loss of sodium, potassium and calcium occurs The acidosis is characterized by low plasma bicarbonate, increased plasma chloride and decreased plasma inorganic phosphorus levels. There is frequently nephrocalcinosis Rickets or osteomalacia is present Weakness as a result of hypokalemia may be prominent

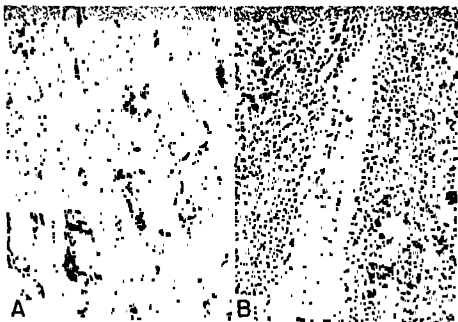


FIGURE 145 RICKETS

Shaft of rib A (x 65) Low power and large numbers of osteoclasts Th power (x 120) of another field to show osteoid on opposite side of trabeculae

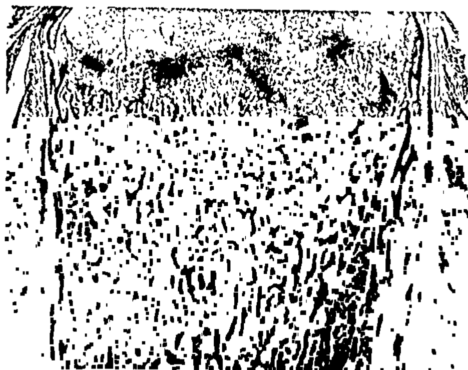


FIGURE 144. RICKETS.
Cartilage shaft junction, rib This shows irregular zone between cartilage and shaft (x 5).

The most important is a syndrome which usually has a hereditary background and makes its appearance in childhood, or in early adulthood.[1309, 1311, 1312, 1313] This disease has received a number of names, "vitamin D resistant rickets" and "phosphate diabetes" have been the most widely used. As might be expected, rickets or osteomalacia are present. Serum calcium values are usually normal; hypophosphatemia is the rule Hyperphosphaturia is present in those cases in which this aspect has been studied. Large amounts of vitamin D, large enough to be in the toxic category, are usually, though not always, effective. This response has led to the term, "vitamin D resistant rickets." It is not entirely clear, however, how the vitamin acts when it is effective. If one believes, as some do,[593] that vitamin D has a specific effect on the resorption of phosphate, the observed therapeutic response makes some sense On the other hand if one does not accept the evidence for a primary action of vitamin D on the function of the kidney tubule, he is left with the feeling that the phosphate diabetes is being helped, though, of course, not always, by toxic amounts of vitamin D, operating in an unexplained fashion. We would



FIGURE 147 HEALING RICKETS

Costochondral junction from a twelve months old child. No vitamin D had been administered prior to hospitalization 1600 U. were given for thirty-two days, followed by 6660 U. to exitus, thirty-seven days later Healing has occurred yet much of the rachitic cartilage is free of inorganic material.

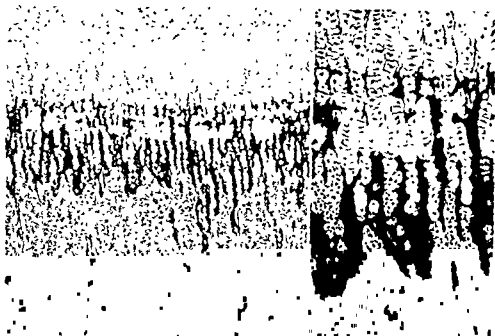


FIGURE 146. HEALING RICKETS

Rib from a nineteen months old baby who had received vitamin D previously. Note dark-staining material (inorganic salts) in cartilage which represents a resumption of the deposition of lime salts at the place they should have been laid down had rickets not been present

The syndrome of "idiopathic hypercalcuria"[152] is poorly understood. It is separated from the above by the absence of acidosis. These individuals have osteomalacia, their main difficulty, however, stems from the presence of renal calculi.

Another extremely interesting syndrome has recently come to light. This is the disease, hypophatasia, which is characterized by low serum alkaline phosphatase levels, hypercalcemia and rickets Exactly how this syndrome is to be so regarded is not clear at this time.[1450]

Excessive losses of calcium may result from profuse perspiration[1318]

The manifestations of rickets as it occurs naturally in man or in animals is no different from what has been described on page 149 in the experimental animal. The initial alteration is a failure of inorganic materials to deposit in the matrix of the costal or epiphyseal cartilages between the rows of hypertrophic cartilage cells[558] This may be a spotty change or may be a diffuse one as if one had rubbed away all of the blue staining material with an eraser. For some as yet unknown reason when hydroxyapatite crystals are not deposited in cartilage matrix something happens to the cells. They are



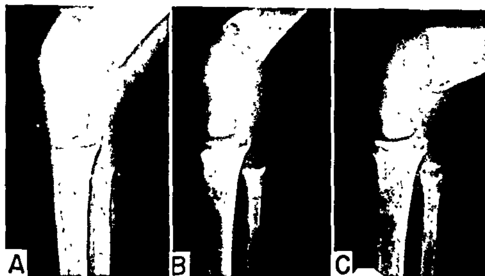


Figure 148. Healing Rickets

Roentgenograms of knees taken on admission (*A*), eleven days (*B*), and thirty-two days (*C*). Therapy with vitamin D had been instituted after *A*. Note filling in of cartilage closing of gap between center of ossification and shaft in *C*.

not destroyed or do not die. At any rate they pile up to many times their normal thickness. Then in time one finds sporadic invasion of the cartilage by "bushes" of capillaries in the vicinity of which calcification may occur. This gives the characteristic stippled appearance in the x-ray.

In the shaft the changes are also as might be expected. Borders of osteoid of varying width are found. The amount of uncalcified bone matrix usually parallels the degree of change at the cartilage-shaft junction. This is not invariable, however. For, if there has been dissociation of activity of cartilage, and of the osteoblast, relatively slight changes may be found in one with pronounced evidence of rickets in the other. Another characteristic of certain cases of rickets is the presence of osteitis fibrosa. By this term we mean excessive bone destruction as evinced by osteoclastic resorption. This is usually an indication of excessive parathyroid activity and may be

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Figure 149. Healed Rickets

(H.L.H. 1049) (x 7). This thirteen month old child had been seen in the hospital three months before death at which time large amounts of vitamin D had been administered because rickets was diagnosed. He returned, dying of lobular pneumonia. Costochondral junction is normal. Deep in shaft is an island of cartilage shown in Figure 150



←



FIGURE 150. ... is COMPOSED OF CELLS IMBEDDED IN AN ...ANT AMOUNT OF UNCALCIFIED MATRIX.

This is interpreted to represent cartilage matrix present at the time therapy was instituted three months before which has not been destroyed or calcified.

→

FIGURE 151. RICKETS

Fanconi Syndrome. Costochondral junction of child dying with syndrome characterized by amino aciduria, phosphaturia, and glucosuria, together with clinical rickets. The skeletal changes are typical of rickets. H and E. (x 7).



FIGURE 153 OSTEOMALACIA

Renal disease A Shaft from rib of child dying of chronic renal insufficiency Note broad borders of osteoid (x 120). *B.* Vertebral body from adult dying of chronic renal insufficiency Osteoid is conspicuous (x 120). *C.* Microradiograph of trabeculi from adult dying with chronic diarrhea Note zone of uncalcified matrix (osteoid) (x 75)



FIGURE 152. RICKETS.

Fanconi Syndrome Trabeculae from shaft of rib shown in Figure 151. Note broad borders of osteoid H. and E. (x 90).

related to the hypocalcemia which may be present. Bone is most usually destroyed but one may also find borders of osteoid which are actively undergoing removal The mechanism for this osteolysis is, of course, not known.

If vitamin D is administered, healing takes place, just as it does in the experimental animal. The site for the deposition of inorganic materials is in the matrix adjacent to the most recently matured cartilage cells. It then spreads to involve portions of the rest of the cartilage. However, much of the hypertrophic region is never calcified and large islands of uncalcified matrix remain and are slowly destroyed.

In the older child, in whom cartilage growth has slowed and in the adult in whom it has ceased altogether, the only manifestation of disease is the presence of osteoid borders about the trabeculae This is, of course, osteomalacia. Little further needs to be said about this.

It would not seem necessary to detail the clinical, biochemical and roentgenological aspects at this time. They are well-covered elsewhere.[1317]

What can be said concerning the incidence of rickets? The geographic distribution of rickets was pointed out years ago by Palm.[1319] When the role of sunlight in effecting the transformation of a provitamin in the skin

One may ask, what is the situation today? Since the entire pattern of disease in childhood has changed, we shall not again have an opportunity to carry out studies, in this country at least, similar to those which were made in Baltimore during the 1930's. Rickets appears to be a disease which is still prevalent in certain parts of the world, for instance Egypt, Turkey and India. Studies in other areas are indicated. Moreover, autopsy studies of children dying of accidents would be most valuable to determine what the skeleton shows in a so-called "normal" group. In this country, osteomalacia on a dietary basis must be very rare. Metabolic osteomalacia associated with renal disease or intestinal dysfunction is common enough. Osteomalacia in other parts of the world doubtless continues to be of importance,[1316] yet its true incidence at this writing is unknown.

Naturally-occurring rickets and osteomalacia in animals, particularly farm species and certain domestic breeds, are common enough, as a few recent references will indicate[1442, 1443, 1444, 1445]

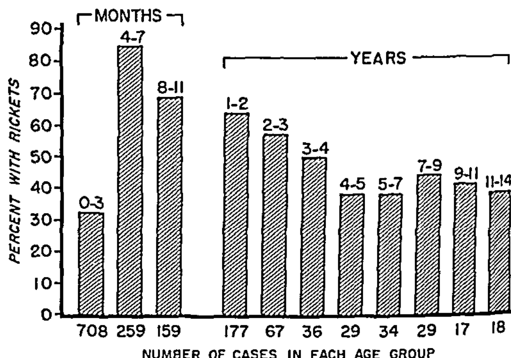


FIGURE 154.

A chart showing the combined prevalence of rickets at autopsy in the Johns Hopkins Hospital series, 1926-1942.

to vitamin D was discovered (page 142), the geographical distribution in relation to ultra-violet radiation was readily explained A number of surveys of the prevalence of rickets have been reported. The classic study is that of Schmorl[1294] which was carried out in Dresden during the period 1901-1908 Schmorl compiled his data from consecutive autopsies on children aged from one month to four years. As is well-known the over-all incidence was found to be high, 94 1 per cent in the 287 children whom he examined from the second to the twenty-fourth month. The difficulties which arise in translating Schmorl's findings to recent studies have been discussed elsewhere.[1448] A summary of the findings in a series of 1533 children studied at The Johns Hopkins Hospital during the period 1928-1942 is presented in Figure 154 The high prevalence is obvious enough.[1315, 1448]

TOCOPHEROL DEFICIENCY

The prominent effects of dietary vitamin E deficiency in the various species, which were discussed on pages 159 to 170, have led to a search for similar changes in the human So, too, lesions which may be produced in experimental animals have prompted therapeutic trials in similar naturally occurring diseases in man This is particularly true of the muscular dystrophies. Unfortunately, all such therapeutic attempts have been uniformly negative and until very recently all efforts to demonstrate tocopherol deficiency in the human have failed.

To Gordon and his associates [1320, 1321, 1322] must go credit for furnishing data which clearly indicate that in newborn babies and in children suffering from certain disease states, the tocopherol content of the plasma may be reduced. Two methods have been utilized to demonstrate this: hemolysis of red blood cells in hydrogen peroxide and chemical determination of tocopherol in plasma These studies have indicated that in both premature and full-term newborn infants the plasma tocopherol levels are low (.26 and 23 mg/100 cc). Following feeding with breast or cow's milk the levels rise.



FIGURE 155 TOCOPHEROL DEFICIENCY

Striated muscle, human (J H H 19731). Section of striated muscle from an infant dying of cystic fibrosis of the pancreas There is extensive necrosis of the fibers (Courtesy of Dr E H Oppenheimer)

SCURVY IN ADULTS

Familiarity with scurvy goes back many hundreds of years We cannot take up the fascinating history of this disease which made so many men miserable on land and on the sea [1336] Scurvy was described among the Crusaders, during the sieges of numerous European cities, such as Breda (1625) and Paris (1870), and as a result of famine, as in Ireland during the last century. With the increasingly long sea voyages beginning at the end of the fifteenth century scurvy came to be the most feared malady of the mariner. The voyages of Vasco Da Gama, Magellan and many others were endangered by outbreaks of scurvy. So, too, explorers of the Arctic and Antarctic suffered grievously from the disease.

How did scurvy manifest itself? The most sensitive area appears to have been the gums. Time and time again one reads of swollen, malodorous gums, which seemed to grow up about the teeth, if such were still present. Even during the present century explorers such as Scott [1337] used the gums as an index to gauge the development of scurvy. During his first Antarctic journey Scott became distinctly alarmed when he learned that his fellow explorer, Shackleton, had "decidedly angry looking gums"

The next important sign of scurvy was pain in the legs, particularly the ankles, though other areas might be involved. The third sign was the appearance of cutaneous hemorrhages, sometimes related to trauma, though on many occasions appearing spontaneously. These hemorrhages might be spotty and located about hair follicles or coalesce to form large ecchymoses. The gingival changes, the pains in the extremities, and the hemorrhagic manifestations were the hallmarks of scurvy. In Cartier's voyage to Newfoundland in 1535 the disease is described as follows, "some did lose all their strength and could not stand on their feete, then did their legges swel, their sinnowes shrinke as black as any cole Others also had all their skins spotted with spots of blood of a purple colour, then did it ascend up to their ankels, knees, thighes, shoulders, armes and necke, their mouth became stincking, their gummes so rotten that all the flesh did fall off, even to the rootes of the teeth, which did also almost all fall out" [1161] Lind, himself, though not so graphic, describes the "putrid gums, the spots and lassitude, with weakness of their knees." [1163]

How might such signs of scurvy in adults be explained? At the end of the seventeenth century two French surgeons described autopsies on soldiers dying with scurvy.[1336] Jean Louis Petit has left the following. "On the bodies of those on whom I carried out a post-mortem examination, I noticed that the periosteum in many places no longer adhered to the bone and that, in several cases, the periosteum was detached from most of the bones, to

The adult level (of healthy hospital personnel) was found to be a convenient means for separating patients with levels above and below .5 mg per 100 cc.

Studies have also been carried out in patients with cystic fibrosis of the pancreas and biliary atresia, two diseases in which faulty absorption of lipids are found In five children with the first disease the plasma levels of tocopherol were low and could be raised by large doses of tocopherol acetate So, too, in two patients with congenital biliary atresia with levels of .09 and zero, respectively, therapy was followed by a rise in tocopherol levels. Hence, it would appear that lesions might be expected to be found, if they were carefully searched for, in children dying from cystic fibrosis of the pancreas or congenital biliary atresia, provided they had not been treated.

Dr. Ella Oppenheimer has allowed us to study such a case which she has recently reported.[1323] This was a twenty-four month old child dying as a result of respiratory infection, associated with cystic fibrosis of the pancreas Evidence was presented for vitamin D deficiency in the form of rickets and for vitamin A deficiency by the presence of metaplasia of the bronchial epithelial cells. Foci of hyaline necrosis of striated muscle fibers with associated inflammatory changes and proliferation of the sarcolemma nuclei were also seen. These alterations are certainly identical with those seen in vitamin E deficiency in experimental animals. Unfortunately, in this case no plasma tocopherol determinations were performed. It is hoped that in the future the skeletal muscles will be diligently examined, particularly when poor fat absorption is to be expected.

SCURVY IN INFANTS

The clinical and pathologic manifestations of scurvy result from a deficiency of ascorbic acid, but, as has been indicated on page 195, the terms ascorbic acid deficiency and scurvy are not necessarily synonymous A number of derangements in physiological and biochemical functions, which are not part of the classical scurvy syndrome in human infants or adults, result from ascorbic acid deficiency. Scurvy, as it is seen clinically or at autopsy, results from a derangement of certain cells fibroblasts, osteoblasts, and odontoblasts, in that they lose their capacity to promote the formation of the fibrous protein, collagen, whether this be in the form of ordinary connective tissue, osteoid or dentine. So, too, abnormalities occur in the blood vessels, which lead to localized hemorrhages.

To Thomas Barlow [1324] must go the credit for making infantile scurvy a clear-cut clinical entity Although others, including Glisson himself, were familiar with scurvy, it remained for Barlow in 1883 to delineate the clinical course of the disease in 32 infants and to describe the gross pathological changes encountered at autopsy These are (1) generalized or localized hemorrhages, particularly subperiosteal, (2) separation of the epiphyseal cartilages from the shafts of the long bones, and (3) swollen bleeding gums, if the teeth have erupted

After Barlow's initial description in 1883 the disease became extremely prevalent in Europe and in the United States This increase appeared to be related to the newly-introduced practice of the pasteurization of milk and to the use of proprietary baby foods A number of able descriptions were presented from the 1890's on by German pathologists Naegeli,[1325] Schmorl,[1326] Schoedel [727] and Fraenkel,[725] [726] Attention centered on the skeletal system, which as a result of its vigorous growth in infancy, exhibits the most marked changes. We shall draw on these earlier observations, as well as on our own material [1327] in the descriptions which follow After taking up the pathologic changes, certain features of the clinical picture will be discussed, followed by a brief résumé of the x-ray findings. Scurvy is frequently associated with rickets; in fact, most of the cases studied by the German pathologists had rickets of varying degree, sometimes extreme We shall comment on the problem of the association of scurvy and rickets at the end of this section

The [illegible] of the organism to form connective [illegible] infancy the growing skeleton is the a[illegible] osteoid) formation is going on most briskly. Thus, changes in the skeleton provide the most telling char-

such an extent that, on making a longitudinal incision of the ribs, I found the latter to be bare, rough and uneven and loose from the cartilage and scarcely attached to the ligaments and tendons of the back part. In some I pulled the bones entirely out of their epiphyses which were kept in place by the ligaments and tendons. This was possible only in the bodies of young recruits, of whom we had a fairly large number at the time." M. Poupart in 1699 describes the clinical picture of scurvy in patients seen in Paris and goes on to say, "On moving these patients to and fro one could hear a slightly grating sound produced by the bones, which was mentioned by Mr N. V., a physician in La Rochelle in his *Traite du Scorbut*, but of which he admitted not to know the real cause. The cause was this: On making dissections of all the corpses in which the sound was heard, I noticed that the epiphyses were completely separated from the bones so that the latter on being rubbed against each other, caused the grating noise."[1336]

Thus the stage was set for the classic descriptions of scurvy in infants which were to come 200 years later, as well as the best modern-day description of scurvy in 23 adults, which was contributed by Aschoff and Koch[730] in 1919 Grossly, the ribs evinced thickening at the costochrondal junctions; in many the cartilage was separated from the spongiosa. The adjacent bone marrow showed hemorrhagic foci. On microscopic examination the cellular marrow was replaced by a loose, mucous-like tissue. In other areas it was more cellular so as to resemble granulation tissue. In the spongiosa the trabeculae lay in all directions. The cortices of the ribs were greatly rarefied In contrast to the ribs, the long bones examined showed very little Aschoff and Koch illustrated a section of the gum. This revealed hemorrhage beneath the epithelium together with hemosiderin-laden cells In this series described by Aschoff and Koch, the subjects, who were soldiers dying in Roumania, appeared well-nourished, so that it would appear that the cases are examples of uncomplicated scurvy.

Scurvy continues to be reported in adults, although the manifestations are not as dramatic as those described in earlier times. A good deal of interest has centered on the role of ascorbic acid deficiency in the development of anemia This had been pointed out by Vilter[1338] some years ago and was recently restudied by Bronte-Stewart.[1452] The anemia which is seen in scurvy appears to be a definite entity which responds to ascorbic acid when no other changes are made in the nutrition of the patient.

Studies of tyrosine metabolism have also been reported in adults with scurvy[1454] When tyrosine was administered, large amounts of tyrosyl derivatives were found in the urine of individuals with scurvy to whom no vitamin C had been administered Capillary alterations seemed to become worse while tyrosine was being administered.

acteristics of scurvy clinically and roentgenologically. So, too, scurvy is most easily recognized at autopsy by its tell-tale marks on the skeleton. The diagnosis can be made grossly by testing the skeleton, so to speak. How easily may the costal cartilage be separated from the shaft and how readily may the periosteum be stripped from the cortex of the rib? These same criteria apply as well to the long bones. If these two features of generalized decreased resistance to mechanical force may be demonstrated, scurvy is almost certainly present. The disease may be more obvious if there is swelling of the costal cartilages, evidence of premortem epiphyseal separations, or subperiosteal hemorrhages of varying degree. How may these gross changes be explained when the bone is examined under the microscope? The picture is similar to that which has already been described in the guinea pig (page 178).

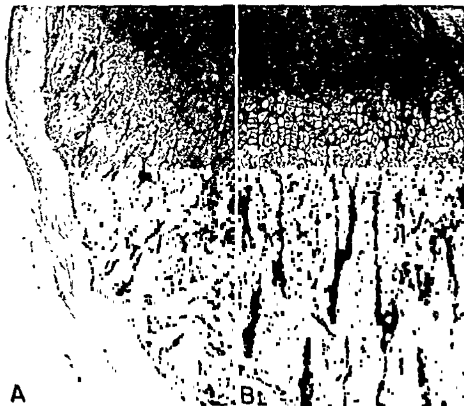


FIGURE 157. SCURVY

Costochondral junction (H.L.H. 1751). *A* and *B*. Lateral margin and cartilage shaft junction shown in Figure 156. *A*. Shows fractures of lattice. *B*. Note lattice devoid of bone upon which spindle shaped osteoblasts are lying.



FIGURE 156. SCURVY.

Costochondral junction, human (H.L.H 1751). A nine month old bottle-fed infant dying of meningococcus sepsis. The cartilage shaft junction is slightly irregular, particularly laterally

bone by the deposition of inorganic calcium and phosphorus in the form of hydroxyapatite. The scorbutic state is characterized by failure of inter cellular substances to be elaborated Osteoid, the organic matrix of bone like collagen and dentine, is an intercellular substance In scurvy there a failure of the osteoblasts to promote the formation of osteoid If th single point is understood the morphologic changes in the skeleton an their clinical and x-ray manifestations should be clear enough.

As the scorbutic state develops in the infant, the cartilage cells of th epiphyseal plate continue to proliferate and arrange themselves in rows i normal fashion. So, too, lime salts are deposited in the cartilaginous matr substance between the columns of hypertrophic cartilage cells. Howeve the next step in the orderly sequence of bone growth is deficient, osteoblas fail to form osteoid on the spicules of calcified cartilage matrix. In additio these spicules are not destroyed Consequently, a wide zone of bare, calc

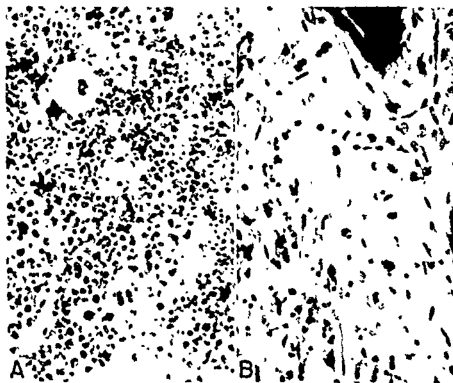


FIGURE 159 SCURVY

Shaft, human (H L H 1161) A Cellular marrow from deep in shaft B Fibrous mar row to show spindle shaped impotent osteoblasts which are found about spicules o

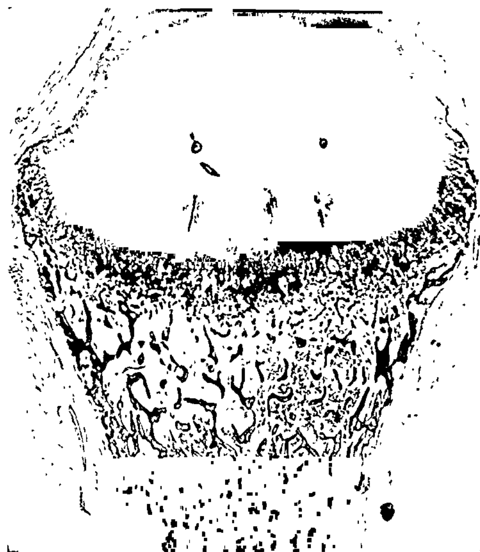


FIGURE 158 SCURVY.

Costochondral junction (H.L.H. 1161). From a five month old child dying of bacillary dysentery Note convex cartilage on concave shaft and disorganized metaphysis

It will be recalled from the discussion of normal osteogenesis on page 145 that growth of the long bones, including the ribs, takes place by a continuous multiplication and piling up of the cartilage cells which form the epiphyseal plates. A deposition of lime salts occurs in the matrix substance in the interstices of the hypertrophic cells at the cartilage-shaft junction. Osteoid tissue is deposited on this frame work of matrix impregnated with lime salts; such bone matrix is immediately converted into true

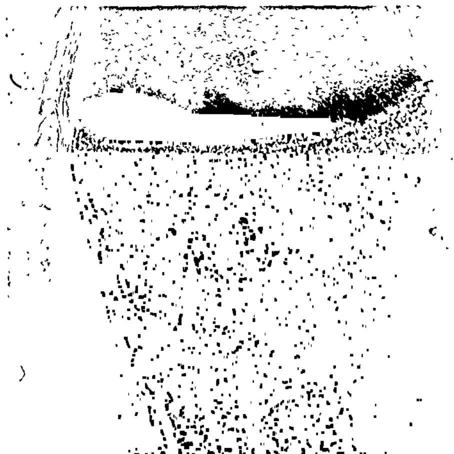


FIGURE 161 SCURVY, HEALED

Costochondral junction (H L H. 1432) Twin of child shown in Figure 160 after treatment for one month Scurvy is healed rickets is present

motion and weight bearing, thus, they are especially liable to fracture The changes which accompany such breaks lead to the characteristic lesions of scurvy in the skeleton

The first site of the appearance of fractures is usually at the periphery of the bone where the cortex and the cartilage are in juxtaposition It is obvious that the most displacement would be present here As the lattice develops in width, an increasingly fragile zone is formed. It is inevitable that partial or complete fractures of the spicules of lattice will occur and that separation and deformity of the cartilage-shaft junction will soon follow. Such fractures of the calcified matrix material lead to a feature of



Figure 160. Scurvy.

Costochondral junction (H.L.H. 1441) Severe scurvy in child dying of burns. Little evidence of healing is present.

fied cartilage matrix is found just beneath the actively growing cartilage plate. Park[1328] has aptly called this formation the "scorbutic lattice," since it is a "lattice" of calcified cartilaginous matrix material devoid of bone. The development of this zone determines the resultant pathologic picture. It must already be obvious that such spicules of calcified matrix material, unencased by bone, are unresistant to the stresses and strains of



FIGURE 161. Scurvy [illegible]

Costochondral junction (H.L.H. 1152) Tw[illegible] ment for one month. Scurvy is healed [illegible]

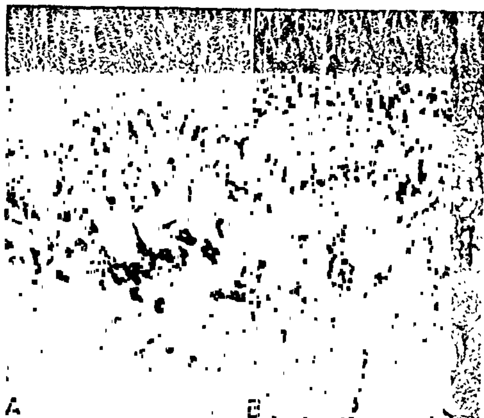


FIGURE 162 SCURVY.

Effect of treatment, human *A*, H.L.H. 1441. *B* H.L.H. 1452. Higher powers of preceding figures to show effects of therapy.

the classical textbook picture of scurvy, which Fraenkel[725, 726] called the "Trummerfeld" or area of debris. Here, beneath the cartilage and its proximal calcified portion, are found spicules of calcified matrix in considerable disarray, lying horizontally and in every other direction About the fractures and in clefts in the matrix a pinkish staining hyaline material is present Large numbers of erythrocytes are also found, together with many cells which can be regarded as impotent osteoblasts; such cells have a stellate appearance and resemble fibroblasts Macrophages containing hemosiderin may also be identified.

Unlike the experimental disease in the guinea pig (page 145), absolute scurvy in the human is very rare, so that evidences of healing are usually encountered. Osteoid and bone are found about some of the fractures; the amount of healing varies from case to case, depending no doubt on the degree and duration of the deficiency state. Beneath the *Trummerfeldzone*

is found an area where there are no hematopoietic cells, thus the marrow appears to be made up of loosely arranged connective tissue cells without many fibers which Schoedel[727] called the *Gerustmark*. The reason for the disappearance of marrow cells, leaving only connective tissue elements, is not clear.

From what has been said it is apparent that the fractures, presence of pink-staining material, hemorrhage, and cellular proliferation are dependent on the development of a structurally inferior zone just beneath the epiphyseal cartilaginous plate. That the stresses and strains resulting from muscle pull and motion are responsible for these classical signs of scurvy at the growing ends of the bone was experimentally shown some years ago in guinea pigs (page 178).

As already noted, evidences of healing are, in our own experience at least, always present in the infant. Such foci of new matrix production and its subsequent calcification about spicules of fractured "lattice" indicate what might be expected if one were to examine the bone from an adequately treated infant some days after the institution of therapy. Some years ago Dr. Park and I had an opportunity to observe a well-controlled experiment of this type in the human. The infants were twins, aged eleven months, who were badly burned when they pulled a kerosene stove over upon themselves. One child died shortly after being admitted to the hospital. At autopsy the costochondral junctions were enlarged, the cartilage and shaft were freely movable upon one another and the periosteum was easily stripped from the cortex. Postmortem x-ray examination of the skeleton corroborated the gross diagnosis of scurvy. On the basis of this the second twin was intensively treated with ascorbic acid. She died a month later of sepsis. At autopsy there was no gross evidence of scurvy. Sections of the costochondral junctions are shown in Figures 160 and 161. Figure 160 shows classical full-blown scurvy. In Figure 161 the disease has healed. In the metaphysis beneath the shaft one finds formations of bone containing irregularly arranged cores of calcified cartilage matrix. These indicate the fracture patterns which were present on admission one month before. The bone about them has been formed during the period of treatment. Incidentally, vitamin D was not administered. Evidence of rickets is present, brought out in part by the vigorous new formation of osteoid following vitamin C therapy (see below).

The salient clinical feature of scurvy is pain in the extremities, particularly the legs. Park[1328] epitomizes this in the words of a mother. "Anyway you lay him is the way he lays. If you lay him on his side he makes no effort to move or nothing. It looks like most of his trouble is from the hips down." In Park's series[1328] and in the group reported by McIntosh[1329] painful extremities were found in over 90 per cent of the cases observed. Hemor-



FIGURE 163. SCURVY, HEALED

Costochondral junction (H.L.H. 244). Costochondral junction from a seven month old white male infant who died as a result of diarrhea and dehydration This was a purely accidental finding at autopsy (see Figure 164).

rhages in the gums were present in a smaller number, but were not found in the absence of teeth. When gingival hemorrhages were present the signs of scurvy were well-developed. Petechial hemorrhages were infrequent in the Baltimore group, 15 per cent of the infants at the Babies Hospital showed ecchymoses *Enlarged costochrondral junctions, as well as swelling of the* ankles or wrists, may be present but the differentiation of scurvy and rickets in these areas is difficult. One point which has been brought out by Park[1328] and which impressed us even more forcibly in our more recent study[1327] was the large number of cases of scurvy found at autopsy in which the disease had not at all been suspected during life. To be sure, a certain number (six) of the sixty-nine infants reported were dead on arrival or dead shortly thereafter.[1334] So, too, a high proportion were extremely ill and attention was directed at combating the disease, so that the manifestations of scurvy were doubtless ignored In only six of the sixty-nine cases showing post-

mortem anatomical stigmata of scurvy was a definite diagnosis of the disease made clinically. One must continue to wonder if cases of scurvy are missed in the clinic today.

It is apparent that anatomical evidence is present before the disease may be recognized by symptoms or on clinical examination Are any other diagnostic tests available? A good deal has been written on the use of ascorbic acid concentrations in blood plasma or white blood cells, it is pretty well agreed that such determinations are of more use in excluding scurvy than in making a definite diagnosis Load tests such as the one developed by Kajdi[1330] would appear to offer more promise, unfortunately this particular procedure, as well as similar ones, does not appear to have gained very wide application Alkaline phosphatase values in the serum are decreased in scurvy[1335] The excretion of hydroxyacids is increased[710, 1451]

The increasing clinical recognition of scurvy in infants and studies of such cases at autopsy coincided with the advent of the roentgenogram. Thus, this new tool was applied to the disease at an early date In 1903, Cassel[1331] described skeletal changes associated with subperiosteal hematomata. Fraenkel[725, 726] correlated the anatomical and x-ray changes in scurvy and called attention to the bright zone beneath the cartilage and the area of rarefaction beneath this His task was difficult because of the concomitant presence of rickets In 1912, Reyher[1372] described the sign in the epiphyses which bears his name Park and his associates[1328] have

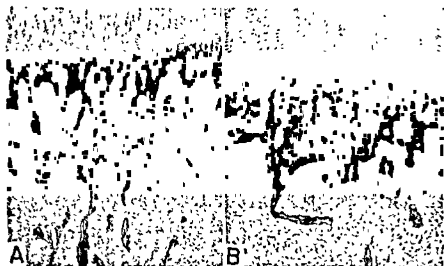


FIGURE 164 SCURVY, HEALED

Costochondral junction, human A and B (H.L.H. 241) Higher powers of costochondral junctions shown in Figure 163 Note fracture patterns of trabeculae.



FIGURE 163 SCURVY, HEALED

Costochondral junction (H L.H 244). Costochondral junction from a seven month old white male infant who died as a result of diarrhea and dehydration This was a purely accidental finding at autopsy (see Figure 164).

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FIGURE 165 SCURVY

Upper tibia (H.L.H 1155). Lesion at lateral margin which shows low outer cortex and lattice have rubbed away

added much by pointing out some of the earliest signs of scurvy in the skeleton and correlating these with the histological alterations which have been described above. In the x-ray one may expect to find changes at the cartilage shaft junction, in the shaft itself and in and about the periosteum, though Park is careful to point out that, "there are no early signs of scurvy" in the x-ray. The history is the most reliable diagnostic point "Every infant, known not to have received antiscorbutic substances over a period of two months, should be held under suspicion." [1327]

The earliest clinical x-ray signs contributed by Park *et al.*[1328] consist of defects at the corners of the ends of the long bones (knees, wrists, ankles). In microscopic sections one can see fractures here when the lattice has been pulled away as well as rarefaction of the cortex, which in some instances may be fragmented.

The question, "How important a disease is infantile scurvy today?", must be asked. One can only cite some of the reports which have been presented in the past from several large pediatric clinics. During the period 1908 to 1934 the total number of cases seen each year in the outpatient clinic of the Babies Hospital in New York City fluctuated from one or two to an all high of over twenty in 1925.[1329] During the decade, 1936-45, forty-one cases of clinically manifest scurvy were observed in every 100,000 outpatient visits to the Children's Hospital in Boston [1333] This was in contrast to an incidence of fifty-eight cases per 100,000 visits during the preceding ten year period. From what has already been said scurvy must be well-advanced before it manifests itself clinically. This was the experience of Park in a study published in 1935.[1328] Further data were presented in 1950 [1327] from an examination of the bones at autopsy of a series of 1,303 children dying in the period 1926-1942. The incidence by month during the two years is shown in Figure 168. If the period from the third through the eleventh month is specifically examined, it is found that, of the 487 cases falling into this age group, sixty-three, or almost 13 per cent, had anatomical evidence of scurvy. In only a few of these, as already noted, was the clinical diagnosis of scurvy entertained, though as far as the clinician is concerned certain extenuating circumstances are obvious: scurvy not far enough advanced, confusion by rickets, severe illness masking pain, child dead on arrival, or all attention given to treating the disease from which the child died.

In this series [1327] a special analysis was made of the feeding history. No cases of scurvy were found in those children who had been breast fed their entire lives or a greater part, even though they had received no supplemental vitamin C. This is in conformity with the observations of Barlow [1324] who stated, "In no single case at the time of onset of the malady has the child

been breast fed" Any correlation of the feeding of cow's milk with the bone lesions at autopsy is difficult because of so many variables

In Table XIII are presented the chief causes of death in the Johns Hopkins Hospital series.[1327] This is of interest in pointing out the toll of infectious disease in this preantibiotic period The number of instances of scurvy, i e, two-thirds of the total, which were associated with a disease of short duration i e, fourteen days or less, would indicate that the disease may have had little to do with the development of scurvy, since in a number skeletal changes were well-marked

The problem of the association of rickets and scurvy has plagued the clinician and pathologist since the very beginning Moller, in 1859, referred to the case he described as one of acute rickets Barlow,[1324] it will be

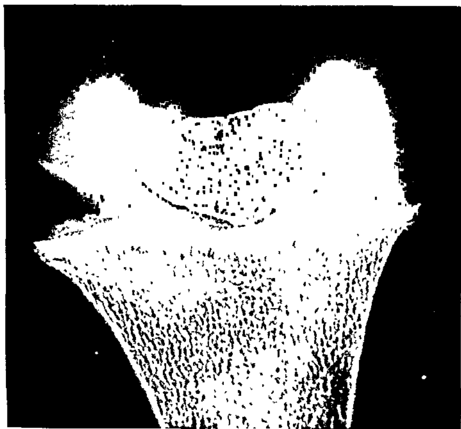


FIGURE 167 SCURVY

Roentgenogram, tibia, x-ray of bone shown on section in Figures 165 and 166. This corner lesion is characteristic



FIGURE 166. SCURVY.

Corner, upper tibia, human Higher power of Figure 165.

TABLE XIII

CAUSES OF DEATH IN 69 CASES OF SCURVY PROVED AT AUTOPSY

Cause of Death	*Number*	*Duration* *Acute**	*Subacute or Chronic***
Lobular Pneumonia	22	13	9
Enteritis	19	14	5
Purulent Menigitis	10	7	3
Tuberculosis	3	0	3
Sudden, With or Without Generalized Hemorrhages	6	6	0
Miscellaneous (Pyloric Obstruction, Retropharyngeal Abscess, etc.)	9	6	3
Total	69	46	23

* Duration 0-14 Days

** Duration 14 Days or More

been breast fed" Any correlation of the feeding of cow's milk with the bone lesions at autopsy is difficult because of so many variables

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FIGURE 167. SCURVY

Roentgenogram, tibia, x ray of bone shown on section in Figures 165 and 166. This corner lesion is characteristic

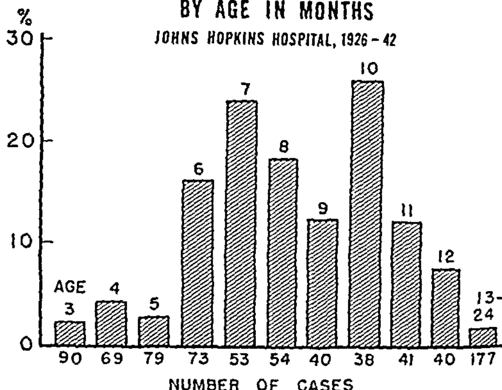


FIGURE 168. PREVALENCE OF SCURVY AT AUTOPSY.

recalled, was able to separate the two diseases on clinical grounds and to point out certain characteristics of scurvy, such as subperiosteal hemorrhages and epiphyseal separation, which set it apart from rickets. However, the pathologists of that time: Naegeli,[1325] Schodel,[727] Schmorl[1326] and Fraenkel[725, 726] studied and reported cases of scurvy in which rickets was a complicating factor. We have had the same difficulties[1453] and have indicated elsewhere[1327] that the prevalence of scurvy in the Johns Hopkins series might have been greater had not rickets been present in so many to obscure the picture. Perhaps we are in a better position today than were the pathologists at the turn of the century, since there is a better understanding of the 2 diseases as a result of experimental studies Both scurvy and rickets are alike in that bone growth must take place if they are to manifest themselves. Thus, they are seen in the same places: middle ribs, knees, wrists, et cetera, those areas where growth is going on most rapidly. But the primary defect in each is entirely different In scurvy the osteoblast becomes impotent to form bone matrix (osteoid); while in rickets the

deposition of inorganic materials in the matrices of cartilage and bone is defective In a group of sixty-nine cases of scurvy which we have studied the absence or presence of rickets was as follows: none, twenty-three, rickets, slight, seven, moderate, thirty-six, and severe, three The small number of instances of severe rickets in this group of cases definitely proved to have scurvy bears out what was mentioned above.

Briefly, it will be recalled that in scurvy growth of the cartilage is not impaired until late in the disease Hence, unless rickets is present and provided the cartilage cells continue to proliferate, inorganic materials may be expected to deposit in the cartilage to produce the tell-tale scorbutic "lattice." But if inorganic materials do not deposit at all or do so in a spotty fashion no lattice will form. Naturally no fractures will be seen if no lattice is present! Hence, it is easy to see how the presence of rickets can completely mask the development of scurvy. One has to find a few fractures of an incompletely formed lattice or the presence of extensive hemorrhages. Treatment of rickets might be expected to bring out the picture of scurvy if the latter disease had been obscured by the presence of rickets We have not encountered this situation However, the opposite has been observed, i e, the unmasking of rickets or an increase in its severity by treatment of scurvy. In such a situation much new bone matrix will suddenly appear and, if inorganic materials to deposit in it are at a premium, such matrix will be deficient in them In the three instances of severe rickets observed in our studies the disease was brought out by the treatment of the scurvy.

THE BERIBERI SYNDROME

The syndrome, which is called beriberi, has been recognized for centuries The derivation of the term beriberi comes from the Sinhalese, meaning "cannot."

A most graphic "general account of the symptoms" is that by Malcolmson which was published in Madras in 1835[1319] "The disease presents such a variety of symptoms that it will be more instructive to consider them in detail, than to attempt any elaborate general description It will be sufficient to describe the most remarkable characters

"It usually commences gradually, with a feeling of numbness, sense of weight and slight weakness and stiffness below the middle of the thighs, sometimes preceded by muscular pains There is slight oedema of the feet and legs, especially along the tibiae, often found to come on after the other symptoms The walk is unsteady and tottering even when the patient is not aware of weakness in the limbs, which are occasionally tremulous, spasms occur in the calves and soles of the feet, sometimes becoming general and occasionally shooting to the chest and larynx, obstructing respiration and speech The want of power often rapidly increases to almost total palsy, especially of the extensor muscles, and in a few cases, the patient after slight indisposition suddenly loses the use of his legs. Rigidity and various affections of the nerves accompany the paralytic symptoms, and there is sometimes pain along the spine, commonly at the two last lumbar vertebrae. In some cases the disease goes no further and a cure is effected; but more frequently, the numbness extends upwards towards the abdomen, there is general sense of lassitude and aversion to motion, and the hands, arms, and chest (and in a few cases even the neck and lips) are gradually benumbed There is oppression and weight at praecordia, dyspnoea on slight exertion, diffused and irregular pulsation in the cardiac region, and the face and hands are puffy and oedematous. The patient is often found dead in bed, or sinks after several fainting fits or throbbings at the heart, or the oedema rapidly increases and extends up the trunk, violent dyspnoea and inability to lie down in bed comes on, with anxiety, cold sweats, cold extremities, rapid feeble pulse, urgent thirst and partial suppression of urine. At the commencement the urine is always scanty, of a deep red colour without cloud or sediment and possessing very peculiar properties, in some old cases it becomes copious, turbid, and pale with a large white deposit, and is passed with pain, from an irritable bladder. The stomach is irritable in many bad cases, and pain and tenderness in the epigastrium is sometimes complained of; there is in a few, pain in the abdomen, or a sense of heat is

diffused over it and the chest. Effusion takes place into the chest and more rarely into the abdomen, and there are now and then some signs of inflammation of the pleura or bronchi. In the early stage, the pulse may be full, hard and frequent or little altered; when the face is puffy and there is weight and oppression at the praecordia it is quick, often irregular and usually small, although it is occasionally strong. Various dyspeptic symptoms occur, the bowels are often costive, the stools green and variously disordered and the eyes often tinged yellow. The skin is rather cold, unless there is pyrexia which is often present in the evening. The disease is sometimes fatal in a few hours, but is often chronic, and in these, the patient is liable to sudden death, to rapid aggravation of the symptoms, or supervention of new and more formidable ones, by which he is soon carried off; and if he survives these, he may live for a long time bedridden, dropsical, and a true paralytic."

Just as pellagra is prevalent in areas where the main dietary staple is corn, so beriberi is encountered where another cereal grain, rice, furnishes the main proportion of calories to the diet. Thus, beriberi has a definite environmental distribution in relation to rice eating. The endemic center has classically been Eastern Asia: Japan, China, the Philippines, Indo-China, Malaya and Java. The disease has also been encountered in certain parts of Africa and South America. In recent years the syndrome has been seen in association with alcoholism in North America.

The history of beriberi provides one of the first clear-cut examples of disease produced by nutritional deficiency. As has occurred in the evolution of our knowledge of other deficiency diseases, such as pellagra, and even pernicious anemia, many theories were postulated concerning the etiology before the correct one was accepted. If one scans the references in the Index Catalog of the Library of the Surgeon General's Office, U S. Army, he may get some insight as to the varied ideas which were advanced concerning the etiology of beriberi. Particularly prominent were: chemical causes (arsenic, oxalate, phosphorus, carbon dioxide, food poisoning) and animate agents (protozoa, helminths, bacteria, fungi). The latter were indicted, as was the trend towards the latter part of the nineteenth century and the first part of this one, when the revolutionary discoveries of Pasteur and others were so much in the forefront.

Certain fundamental observations which were made from the 1870's on, focused attention on the relationship of rice to beriberi. It should be pointed out that in the rice eating countries, beriberi had occurred only sporadically until the latter part of the nineteenth century. Then, with the advent of steam-powered mills, which ground the husks from rice so efficiently, the prevalence of the disease rose rapidly, particularly in prisons and military establishments. The Dutch and Japanese were especially inter-

ested in the latter areas; consequently the main advances came from investigators from Holland and Japan.

In 1873, Van Leent, a Dutch naval doctor, noted a difference in morbidity and mortality from beriberi among the Indian and European sailors of the Dutch navy He ascribed this to differences in diet. That of the Indians consisted mainly of rice; the Europeans partook of a more varied fare, particularly with respect to protein and fat [1340]

By the 1880's, Takaki,[1341] a Japanese medical officer, had begun to study beriberi which had come to be recognized as a serious problem in the navy Takaki had only to compare the Japanese sailors who had developed beriberi with the men of the British Navy who did not suffer from the disease. The reason for the difference was clear: a diet in which rice predominated versus a diet in which there was plenty of meat and vegetables So, too, Takaki noted a disparity between Japanese officers and the common sailors, the rice diet of the former group was supplemented with meat and vegetables Takaki in a fashion similar to that of Lind over a hundred years earlier was able to convince his superiors that meat, vegetables and condensed milk should be introduced to supplement the rice. When this was accomplished beriberi became virtually extinct in the Japanese Navy

At the same time Christian Eijkman,[778] who was working in Batavia, was able to produce a "beriberi-like" disease in fowl by feeding a diet of polished rice (page 197) He found, too, that the feeding of unpolished rice failed to reproduce the disease. He further suggested that a similar relationship of polished rice consumption to beriberi might exist in the prisons; it did Eijkman isolated from rice polishings a water or alcohol soluble extract which cured the disease in chickens He thought, however, that he was dealing with an intoxication, i e, that rice produced in the intestine a poisonous substance to which the outer layers of the grain were an antidote

It was for Grijns[1342] to postulate that beriberi was the result of a dietary deficiency From here on the story is familiar enough. The isolation by Funk from rice polishings of a potent extract which chemically had the properties of a pyrimidine base led to the introduction of the term "vitamine" During the ensuing years, extracts of increasing potency were prepared, until in 1926, Jansen and Donath isolated a crystalline material. The story reached its climax in 1936 when Williams and his associates announced the structural formula and synthesis of thiamine.

Thus the disease, *beriberi*, came to be intimately connected with a single essential nutrient, thiamine So much so, that in the text books of biochemistry and of medicine it has been customary to discuss beriberi under the headings, "Thiamine" or "Thiamine Deficiency." We feel such an ap-

diffused over it and the chest. Effusion takes place into the chest and more rarely into the abdomen, and there are now and then some signs of inflammation of the pleura or bronchi. In the early stage, the pulse may be full, hard and frequent or little altered; when the face is puffy and there is weight and oppression at the praecordia it is quick, often irregular and usually small, although it is occasionally strong Various dyspeptic symptoms occur, the bowels are often costive, the stools green and variously disordered and the eyes often tinged yellow. The skin is rather cold, unless there is pyrexia which is often present in the evening. The disease is sometimes fatal in a few hours, but is often chronic, and in these, the patient is *liable* to sudden death, to rapid aggravation of the symptoms, or supervention of new and more formidable ones, by which he is soon carried off; and if he survives these, he may live for a long time bedridden, dropsical, and a *true paralytic.*"

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has never deserved the title of 'antineuritic vitamin,' and has not yet shown itself capable of filling completely the role that was formerly assigned to the hypothetical antiberiberi vitamin"

It is important to realize that even today we rely on the pathologic studies which were made during the early years of this century for an understanding of the pathologic anatomy of beriberi. It is further important to understand that beriberi in those days was looked upon as an intoxication or an infection These ideas may have colored the interpretation of the lesions by the pathologists of that era Unfortunately, no recent studies of the neurological changes in cases of endemic beriberi have been carried out. A number of questions remain unanswered. Do specific lesions occur in the peripheral nerves which are unassociated with inanition?[1349] What are the lesions of cerebral beriberi? If such are present, are they similar to those characteristic of the Wernicke syndrome (page 415)? What, if any, is the relationship of other deficiencies to beriberi? Do the lesions which are associated with pantothenic acid and pyridoxine deficiencies of swine have any counterpart in human beriberi? What is the reponse of the neurological changes to therapy?

As already noted, one of the cardinal manifestations of the beriberi syndrome has been heart failure.[1351, 1352] In the Orient evidences of beriberi heart disease have included palpitation, pain, dyspnea, dependent edema, and increasing signs of cardiac enlargement Failure of the right ventricle is manifested by an enlarged and tender liver with increasing ascites, hydrothorax and hydropericardium Definite, though non-specific, electrocardiographic changes were also found This picture was described in beriberi all through the early part of this century. More modern descriptions of Oriental beriberi such as those by Wenckebach[1353, 1354] and Keefer[1355] have tended to draw attention to the possibility that beriberi heart disease might be found in this country. Weiss[1356] in Boston was largely responsible for popularizing Occidental beriberi heart disease In 1940, one case was found for every 160 admissions to the Boston City Hospital Most of the individuals displaying this form of heart disease were alcoholics. The cardiovascular abnormalities were ascribed to thiamine deficiency Various manifestations were observed, such as: orthopnea, asthma, pulmonary edema, reduced vital capacity, gallop rhythm, and increased venous pressure. The cardiac output was raised. Thiamine was effective in therapy only in some of these cases At autopsy, "hydropic change" was found in the myocardial fibers

Further study of beriberi heart disease by Blankenhorn[1359] has led to the following diagnostic criteria (1) enlarged heart with normal sinoauricular rhythm, (2) dependent edema, (3) elevated venous pressure, (4) peripheral neuritis or pellagra; (5) non-specific changes in the electrocardiogram, (6) lack of other recognized cause of heart failure, (7) grossly

proach places undue emphasis on thiamine deficiency in the pathogenesis of this disease and fails to stress that beriberi is a multiple deficiency syndrome We have deliberately chosen as the title for this section, "The Beriberi Syndrome," in order to call attention to this important fact.

Hence, in the present state of our ignorance concerning the pathogenesis of beriberi, definitions such as the following are not satisfactory. "Beriberi may be defined as a nutritional disease due primarily to a deficiency of thiamine (vitamin B_1) and characterized by peripheral neuritis, myocardial changes with congestive failure and edema,"[1343] or "Beriberi is a deficiency disease characterized by multiple neuritis, changes in the cardiovascular system and, frequently, edema. The primary manifestations are attributable to thiamine lack."[1344]

Beriberi, as seen clinically, has been divided into three main types: (1) the dry or typical form; (2) the cardiac or acute pernicious form, and (3) the wet or edematous form. As might be expected there may be mixture of these three main types.[1351, 1352]

The first form, dry beriberi, manifests itself primarily as a nervous system disturbance with hyperesthesia. There is loss of various reflexes and finally paralysis Tenderness and cramps of the muscles of the extremities are prominent There may be numbness of the lips and tremor of the tongue As the disease progresses, marked atrophy of the muculature, particularly that of the extremities, is found. Contractures occur in the end stage.

Cardiac beriberi, or the acute pernicious form, is characterized by sudden and devastating heart failure which may have a fatal course in a matter of hours, or it may be more slowly progressing, giving rise to classical signs of congestive heart failure.

The third classical form, wet beriberi, is characterized by extensive anasarca This may in part be the result of cardiac failure and in part due to hypoproteinemia.

From these clinical manifestations of the disease one might expect to encounter profound changes in certain areas, e g., the nervous tissues and heart, at autopsy. The earlier studies of Baelz,[1345] Scheube,[1346] Wright,[1347] and Durck[1348] revealed degeneration of the peripheral nerve fibers exemplified by various stages of loss of myelin and axoplasm. Changes of the dorsal root ganglion cells and tract degeneration of the columns of Goll in the spinal cord were also observed To what can such changes be ascribed? Unfortunately the answers to these questions are not known. Hence it is impossible to do more than point out our ignorance with a plea for fresh studies on clinical cases and autopsy material. As far as the relation of thiamine to polyneuritis is concerned a statement made by Meiklijohn[1350] in 1940 is perfectly valid today. "When the literature of the last ten years is considered in perspective, the conclusion is inescapable that thiamine

ized at autopsy by scarring of the mural endocardium and the myocardium of the right and/or left ventricles with failure of either/or both chambers A further complication is adherence of either or both atrioventricular valves to the ventricular walls, thus producing incompetency. The reason for this peculiar incidence in Uganda is not clear At the present time nutritional deficiency can be regarded as only one possible etiological factor, anemia, animate agents, et cetera, may be implicated.

Exact figures as to the prevalence of beriberi in various areas today are few. However, the Philippine experiment[1363, 1364] necessitated the formulation of a base line of morbidity and mortality statistics which are of interest In 1947, before the rice enrichment program began, the disease claimed 24,296 lives with a rate of 132 per 100,000. The year before the death rate was 148, while that of tuberculosis, the leading cause, was 170 per 100,000 Clinically, in the Bataan survey, of 12,384 persons examined, 1,580 cases of "frank" or "suspected" beriberi (12.7 per cent) were found

The prevalence of beriberi today among the rice eating population of the world is not known. Since one-half of the world lives on rice, beriberi may be of great importance, though how important we may not know until pointed surveys are carried out.

deficient diet for at least three months, and (8) clinical improvement with reduction of heart size after specific treatment, i.e., thiamine.

From the above one is struck most by criterion number 6, "lack of other recognized cause of heart failure." It would appear this has opened the door to the use of beriberi heart disease as a sort of catch-all in which to throw non-diagnosable types of heart disease, doubtless of varying etiology.

It is unfortunate that the anatomical alterations in the heart accompanying the beriberi syndrome are so equivocal. Earlier observers, such as Durck,[1348] noted inflammatory foci and scarring. Wenckebach[1354] described edema. Weiss[1356] thought that "hydropic degeneration" was a part of the pathologic picture. It is apparent that there is complete lack of any consistent pathognomonic lesion at autopsy. This is disappointing in view of the constant and easily reproducible changes which may be found in the thiamine-deficient animal whether it be rat, mouse, dog, swine, or monkey (page 198). We have been on the lookout for changes for some years but have seen no material that is acceptable. Beriberi heart disease continues to be described.[1358] A recent series of cases was characterized by hypertension.[1357]

To further complicate the status of the myocardium in relation to nutritional factors certain syndromes and autopsy findings therein must be mentioned. For instance, a type of "nutritional heart disease" has been described in the malnourished Bantu of South Africa.[1360] These individuals partake of a diet consisting almost exclusively of a porridge of highly refined maize, white bread, tea, and sugar. The syndrome is characterized by a generalized edema, enlargement of the heart, a hypokinetic circulation, and gallop rhythm. Electrocardiographic changes of a non-specific type are present. Plasma protein concentrations are only slightly reduced (7.1 gm. per cent to 6.1 gm. per cent) and no relation of their levels was found to the anasarca. When the patients were placed on an adequate diet, the abnormal signs and symptoms cleared up unless the disease had progressed into an irreversible stage.

At autopsy[1361] the heart was hypertrophied, weighing as much as 750 grams. None of the usual causes for such enlargement were found. In five of the twelve reported autopsies fresh mural thrombi were observed. Intracellular edema was present though no hydropic change was seen. Slight fibrosis with cellular infiltrations was found in a few of the hearts but such changes were not particularly pronounced. Evidence of liver disease, such as fibrosis and hemosiderosis, was found in most of the cases. The exact etiology is totally obscure, though there appears to be little doubt that faulty nutrition plays a role.

Another form of heart disease, "endomyocardial fibrosis," has been reported from Uganda in central Africa.[1362] This group of cases is character-

INFANTILE BERIBERI

A syndrome characterized by anasarca, dyspnoea, cardiac disturbance, gastrointestinal derangement, oliguria and aphonia was first described in Japan by Hirota in 1898.[1376] The cases which he observed occurred in infants who were being nursed by mothers who had symptoms of beriberi. He found that such children recovered when they were taken from the breast and concluded that the disease was caused by an intoxication

The syndrome was studied extensively in the Philippines, where Vedder[1352] and others observed it and where it accounted for a large number of deaths during the first year of life Vedder describes the typical acute case as follows· "A young infant of from one to three months of age who has always apparently been healthy and well nourished is suddenly seized with paroxysms of pain During such a paroxysm the child straightens out his body and becomes quite rigid, the abdominal walls and epigastrium are tense and hard. He cries constantly, and gradually the face becomes cyanotic and the veins of the neck are engorged. The child acts as if in great pain The pulse is very small and rapid, and the heart flutters. This attack passes off only to be repeated by others of a similar character at intervals of greater or less length and the child dies anywhere from a few minutes after the beginning of the seizure to ten to twelve hours."

A more chronic form was less common This was characterized by gastrointestinal disturbances such as constipation and vomiting, by anemia, dyspnoea, oliguria and aphonia. The latter sign must be most extraordinary to witness It has been described vividly as follows. "The baby seems to be crying, but because of the loss of voice no sound comes from him, and only his grimaces and twitching of his face offer evidence of the sufferings he is undergoing" Relatively little indication of neurological involvement is seen in either the acute or more chronic forms.

At autopsy, cardiac hypertrophy has been the most prominent finding, with congestion of the viscera Microscopic studies have been few and poor

Vedder noted a dramatic response to rice bran extract, this further convinced him that the disease was truly beriberi.

In recent years this syndrome has continued to be observed in those parts of the world where polished rice is the main dietary staple.[1377, 1378, 1379, 1380, 1381] Little is known of the biochemical and anatomical alterations which accompany the clinical manifestation just mentioned Further studies in areas where the disease continues to be endemic are much needed

THE WERNICKE SYNDROME

In 1881, Wernicke[1365] delineated a syndrome, "acute hemorrhagiche Polioencephalitis superior," which was characterized clinically by clouding of consciousness, ophthalmoplegia and ataxia. At autopsy, symmetrical lesions were found in the gray matter of certain areas of the brain, being particularly prominent in the region of the third ventricle and corpora quadrigemina Two of the three cases Wernicke described were associated with alcoholism; the third followed gastric disturbances which developed after the ingestion of sulfuric acid.

In the ensuing seventy-five years the clinical aspects of this syndrome have been redescribed and elaborated upon many times So, too, extensive correlative pathologic studies have appeared As a result of these clinicopathologic investigations and of experimental observations already mentioned (page 204), the Wernicke syndrome, or Wernicke's encephalopathy, is now considered to result from nutritional deficiency.

As more cases have been observed certain clinical aspects of the syndrome have been amplified,[1366, 1367] though clouding of consciousness, ophthalmoplegia and ataxia remain the foundation Drowsiness may usher in the disease, and is then followed by coma The latter may alternate with stages of excitement and delirium When these are not too marked one may observe symptoms of the Korsakoff type; disorientation in space and time, confabulation, visual and aural hallucinations, et cetera. In fatal cases of the Korsakoff syndrome it is unusual not to find the pathologic lesions described by Wernicke[1368] at autopsy Impairment of vision is usually prominent, fundal hemorrhage may be present Evidences of oculomotor disturbances are most frequent and important. Such consist of irregularity and inequality of the pupils, Argyll-Robertson pupils, paralysis of conjugate eye movements, diplopia, strabismus and nystagmus Polyneuritis may be present. The total protein content of the cerebrospinal fluid may be elevated

At autopsy, changes in the brain are particularly interesting because of their selective and bilateral distribution[1369] Vascular alterations are prominent, these consist of dilatation of small vessels with proliferation of their endothelial cells Perivascular hemorrhages may or may not be prominent. The parenchyma shows necrosis of neurons and degeneration of their processes In time a reaction of glial elements is found The microglia swell and acquire fat droplets, the astrocytes proliferate. Otherwise, little inflammatory reaction is found. The commonest sites of damage are the mammillary bodies. Changes are prominent, though less consistent, in other hypothalmic nuclei, the nucleus parafascicularis and in the medial portion of the

is probably a combination of several nutritional deficiencies affecting the nervous system and need not necessarily be complete in any case" Since the ophthalmoplegia responded to thiamine, a deficiency of this vitamin was thought to be the cause of such paralysis The administration of thiamine improved the polyneuritis, if present. The Korsakoff syndrome did not respond to thiamine. Of Jolliffe's series of twenty-seven cases one-third exhibited evidences of "nicotinic acid deficiency encephalopathy" (see page 326), others had stomatitis and dermatitis thought to be associated with nicotinic acid deficiency Jolliffe[1372] and others, for instance Riggs and Boles,[1373] have indicated that nutritional deficiency forms the basic background for Wernicke's Disease. Thus, a multiple deficiency state rather than a lack of thiamine alone is likely responsible It is, therefore, to be deplored that the hypothesis that uncomplicated thiamine deficiency is the cause of Wernicke's Disease has been rather widely accepted without a full evaluation of the experimental data upon which such a supposition is based

In the pigeons, which were reported by Alexander *et al*,[797] lesions are said to occur in the brain and to consist of symmetrical foci of damage in the gray matter together with hemorrhages and changes in the blood vessels Because of the similarity of these alterations to the pathological manifestations of Wernicke's disease in man, Alexander has concluded that the



FIGURE 170 WERNICKE'S DISEASE

Brain A, and *B* Higher powers of section shown in Figure 169 Proliferation of glial and endothelial cells together with alterations of neurons are shown

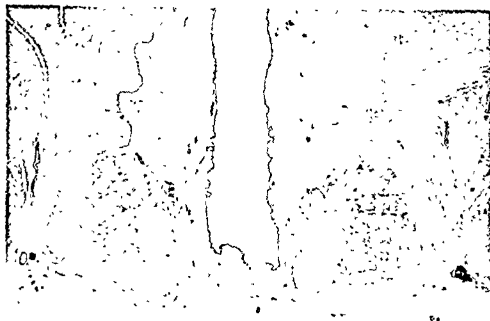


FIGURE 169 WERNICKE'S DISEASE.

Brain Low power through *mammillary bodies from alcoholic dying in coma* (A F.I.P. 97952).

thalamic nuclei. In the midbrain the following may be involved: periaquaductal gray-matter, oculomotor nuclei, posterior colliculi and central gray matter at the junction of mesencephalon and diencephalon. In the hindbrain the gray matter of the floor of the fourth ventricle may be involved. The optic nerve also can evince damage. So, too, changes have been described in the spinal cord,[1310] lesions of the cerebral cortex are uncommon

Early descriptions of this syndrome stressed the role of alcohol in its pathogenesis, though it may be recalled that one of Wernicke's original three cases was *not* an alcoholic. A significant advance was made when several publications pointed out the occurrence of the disease in non-alcoholics,[3367, 1371] whose nutritional status had been interfered with by chronic gastric ulcer, gastritis, carcinoma of the stomach, enteritis, et cetera. At the same time, too, the observations of Alexander[797] in birds (page 206) and of others in rats,[784] cats,[781, 798] and foxes[774] focused attention on the possible nutritional etiology of the disease. All of this led Jolliffe *et al*,[1372] to review twenty-seven cases of Wernicke's syndrome which had been observed at Bellevue Hospital during the period 1935-1940. Since various methods of treatment had been employed, Jolliffe and his co-workers were able to conclude that "the syndrome as originally described by Wernicke

PERNICIOUS ANEMIA

In the years preceding the publication of his classic *On the Constitutional and Local Effects of Disease of the Suprarenal Capsules*, Thomas Addison had already noted (1849) a "remarkable form of general anemia, occurring without any discernible cause whatever"[1362] Later he was to describe the clinical course of this disease as follows.

"It makes its approach in so slow and insidious a manner that the patient can hardly fix a date to the earliest feeling of that languor which is shortly to become so extreme. The countenance gets pale, the whites of the eyes become pearly, the general frame flabby rather than wasted, the pulse perhaps large, but remarkably soft and compressible, and occasionally with a slight jerk, especially under the slightest excitement, there is an increasing indisposition to exertion, with an uncomfortable feeling of faintness or breathlessness in attempting it, the heart is readily made to palpitate; the whole surface of the body presents a blanched, smooth, and waxy appearance, the lips, gums, and tongue seem bloodless, the flabbiness of the solids increases, the appetite fails, extreme languor and faintness supervene, breathlessness and palpitations being produced by the most trifling exertion or emotion, some slight oedema is probably perceived about the ankles, the debility becomes extreme, the patient can no longer rise from his bed, the mind occasionally wanders, he falls into a prostrate and half-torpid state, and at length expires. nevertheless, to the very last, and after a sickness of several months' duration, the bulkiness of the general frame and the amount of obesity often present a most striking contrast to the failure and exhaustion observable in every other respect"[1363]

At autopsy, Addison found virtually nothing, save extreme fatty changes in the heart

After the publication of Addison's first contribution in 1849, the syndrome had begun to be further delineated, for in 1851 Barclay[1364] described two cases of "Death from anaemia," in one of which glossitis was present. In 1872, quite unaware of Addison's publication, Biermer[1365] reported from Zurich a series of fifteen cases of "progressive perniciose Anaemie," which clinically appeared to correspond to the case histories detailed by Addison. In 1876, the appearance of the bone marrow at autopsy was reported on by Cohnheim,[1366] who, because of the hyperplasia and presence of many nucleated red cells, felt that this tissue was the primary site of the disturbance. A year later Samuel Fenwick[1367] delivered three lectures, "On Atrophy of the Stomach" He clearly appears to have grasped the association of gastric atrophy and that form of anemia described by Addison. Fen-

two diseases are identical and further inferred that the latter syndrome results from thiamine deficiency. It will be recalled (page 206) that the diet employed by Alexander consisted of rice, fortified only with riboflavin, ascorbic acid, and sources of vitamin A and D, a ration grossly deficient in certain elements and vitamins, especially those of the B complex and fat-soluble group, particularly vitamin K. It is unfortunate, therefore, that thiamine has been indicted as the sole cause of such lesions in man and pigeons. Alterations of the brain in Chastek paralysis of foxes have also been called a "counterpart" of Wernicke's disease in the human.[797] Thiamine cures the fox syndrome, though it will be recalled that animals which die provide at autopsy some evidence that deficiencies in other nutrients are present as well. Studies in the monkey,[790] which have been described elsewhere (page 206), have failed to reveal the vascular lesions, particularly the hemorrhages, which are so prominent in man. However, it would appear that such differences may not be without explanation. One has only to recall the occurrence of liver disease in alcoholics and the relation of this organ to the production of prothrombin to wonder whether the hemorrhages which are said to be pathognomic of Wernicke's Disease may in any way be related to vitamin K deficiency.[696] It should further be pointed out that the prothrombin time is increased in choline-deficient dogs, whose livers contain large amounts of fat.[989]

From the data so far accumulated in human cases which have been treated with thiamine little doubt exists that the ophthalmoplegia is related to a specific lack of this vitamin.[1374] So, too, the nystagmus and ataxia may be related to thiamine deficiency, though the evidence for this is less conclusive. The cause or course of the mental disturbances are not at all clear at the present time. It is important to realize that those patients, who clinically evince the disease, and are treated and respond rapidly, must not have the anatomical lesions shown in Figures 169 and 170. Rather one must assume that they have "a biochemical lesion" similar to that described by Peters in the experimental animal.[757]

Finally, a recent contribution of Neubuerger calls attention to the decreasing incidence of lesions of the Wernicke type in the chronic alcoholic.[1375] More common is a non-specific degeneration of the granular layer of the cerebellum.

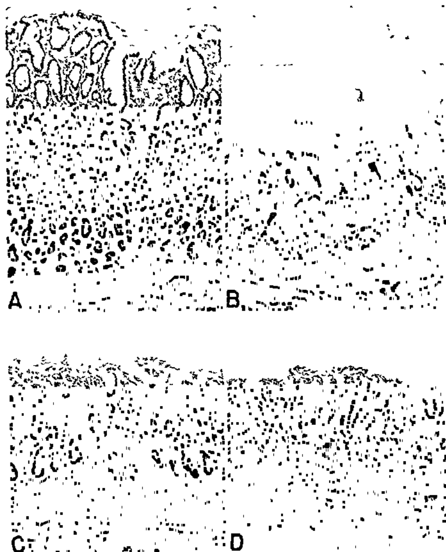


FIGURE 171 PERNICIOUS ANEMIA

Stomach A Normal, *B* (J H H 6795), C (J.H H 8939); *D* (A F.I.P 693355) All x 35 The decrease in width of the glandular layers is clearly seen, as are inflammatory cells in mucosa.

wick anticipated the importance of the stomach in the pathogenesis of pernicious anemia by fifty years. He described the absence of digestive ferments as follows. "I scraped off the mucous membrane of the stomach, and made an infusion of it with distilled water. To two ounces of this infusion was added half drachm of hydrochloric acid A cube of hard boiled albumin of egg was suspended in this mixture and was digested in a water bath at blood heat for nine hours. At the end of this period the albumin was slightly softened on the surface, but its weight was not lessened" Moreover, *finding no cause for anemia developing in a physician, Fenwick* confidently made the diagnosis of atrophy of the stomach before death; autopsy confirmed this! The picture of pernicious anemia as we know it today was completed by Lichtheim [1388] in 1887 when the spinal cord changes were described.

During the period 1855 to 1900 a number of theories concerning the pathogenesis of pernicious anemia were introduced. Not much attention was paid to the primary role of the stomach as proposed by Fenwick.[1387] The two main schools, German and British, looked upon the pathogenesis of pernicious anemia in different ways. The first group thought it was due primarily to a disturbance in the function of the bone marrow with a reversion of this tissue to an embryonal type (Cohnheim [1388]). Ehrlich [1389] had already called attention to the megaloblasts. On the other hand the English clinicians favored the digestive system as the important factor in the development of the disease This hypothesis was championed by Hunter [1390] *who made much of the evidence of hemolysis which seemed to be* present in some cases of pernicious anemia, i e., the jaundice and the presence of iron pigment in the liver. He postulated that the bacteria and their products in the intestinal tract led to an hemolytic anemia

These controversies cannot detain us here.[1391] For the current thought at the turn of the century Hunter's monograph [1390] will be interesting to consult. Before continuing, however, a most prophetic statement made in 1878 by Wernich [1392] should be quoted: "Like beriberi, scorbutus, chlorosis, etc , it (pernicious anemia) belongs to a class of constitutional diseases brought about by disturbances of nutrition."

Today, as a result of liver and other therapy, the natural history of the disease has been completely changed Therefore, we should like to present the clinical and morphological manifestations as they were prior to 1926 We have been fortunate in having for study cases dying of pernicious anemia at the Johns Hopkins Hospital before the introduction of liver.

The clinical picture of pernicious anemia is usually clear-cut. The presenting symptoms may be related to the glossitis, to the general effects of the anemia or to the nervous system. Either one of these manifestations may come first and not be followed by the others for some time. Certain

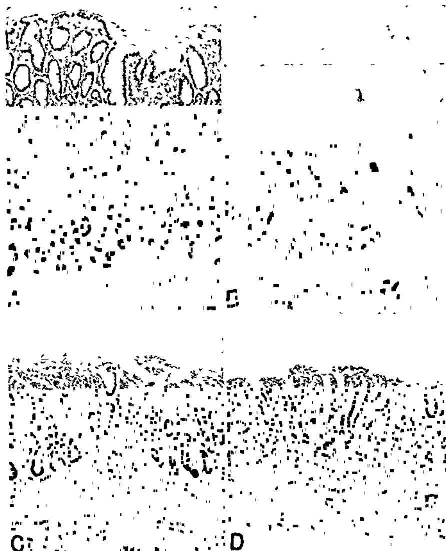


FIGURE 171 PERNICIOUS ANEMIA

Stomach *A* Normal, *B* (J.H H. 6795), *C* (J H H 8939), *D* (A F I P 693355) All x 35 The decrease in width of the glandular layers is clearly seen, as are inflammatory cells in mucosa



FIGURE 172 PERNICIOUS ANEMIA

Stomach. *A* Normal (x 125), *B* (J H.H 8939). Note difference in type of epithelial cells *C* Normal (x 300), *D* (J H H. 6795). Epithelial cells do not resemble those of normal gastric mucosa.

examinations, particularly the blood and gastric contents, are of utmost importance in making the diagnosis. The peripheral blood shows a macrocytic hyperchromic type of anemia with marked poikilocytosis and anisocytosis. Nucleated red blood cells and even megaloblasts may be present (see below). There is leukopenia and usually thrombocytopenia. Examination of the bone marrow[1393 1394] reveals an increase in the percentage of erythropoeitic elements with numerous nucleated forms, particularly prominent are large cells with reticular nuclei and basophilic cytoplasm Such cells are called megaloblasts and today are considered by the majority of hematologists to be abnormal forms The gastric contents almost always show no free hydrochloric acid. As has been stressed many times,[1395 1396] when free acid is present, the diagnosis of Addisonian pernicious anemia should be seriously questioned. As has already been noted, pepsin activity is usually lacking

Besides the glossitis, which is such a prominent part of the disease syndrome, other symptoms relative to the gastrointestinal tract may be present. Diarrhea is a common complaint and may be severe This, of course, may lead to a loss of other essential nutrients No evidence of impaired hepatic function can usually be detected even though clinical or serum jaundice may be present Such pigment is related to increased blood destruction Further evidence of hemolysis is the presence of increased urinary and fecal urobilinogen concentrations. It might further be mentioned that the incidence of carcinoma of the stomach is higher in individuals suffering from pernicious anemia than in similar age groups[1397]

The hereditary nature of pernicious anemia appears to be well-established. So, too, are the racial predilections The disease is rare in the negro and in certain other races

The earliest clinical manifestations of nervous system involvement relate to sensory disturbances paresthesias and loss of deep sensation. These symptoms and signs begin in the lower extremities, as time goes on the hands are likewise affected Later, evidences of motor involvement make themselves apparent In some cases the neurological involvement mimics a true peripheral polyneuritis In addition to such evidence of peripheral and spinal cord involvement, manifestations of cerebral derangement indicate that the brain itself may be affected.

The impressive observations which Fenwick[1381] made on the stomach eighty years ago have already been mentioned. The gross appearance of the fundus of the stomach, flattening of the rugae with thinning and transparency of the mucosa, belies its microscopic appearance The latter is usually fairly well-preserved as a result of the anacidity and absence of gastric enzyme activity

Microscopically, the epithelium is composed of mucous glands, rather

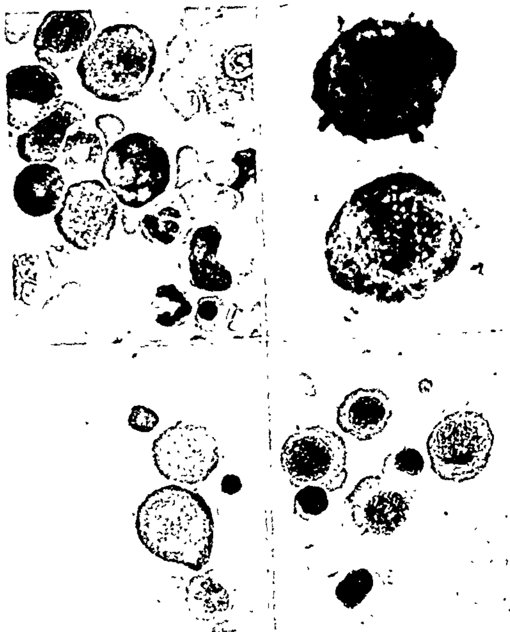


FIGURE 173. PERNICIOUS ANEMIA.

Bone marrow. Series of megaloblastic cells from sternal marrow of patient with pernicious anemia who has not received any therapy (courtesy of Dr. R. J. Lukes).

resembling those present in the small intestine. In the severe case no trace of chief or parietal cells can be found. In what represents the submucosa, aggregations of small glands, some of which contain mucus, can be found. Sometimes dilated structures resembling the glands seen in cystic gastritis may be present. From case to case one finds varying degrees of cellular infiltration, usually of the lymphocyte and plasma cell varieties. It is felt by some[1398] that this appearance of the stomach is specific, however, we would find it difficult to make the diagnosis of pernicious anemia on the gastric changes alone.

Grossly, the glossitis observed in pernicious anemia is no different from that observed in iron deficiency (page 299), pellagra (page 319) or experimentally-produced nicotinic acid (page 220), pyridoxine (page 248) or biotin (page 266) deficiencies in the human. The end result is a smooth shiny tongue whose papillae all appear to have disappeared. Microscopic examination confirms this. The epithelium is greatly reduced in thickness. The primary and secondary components of both the filiform and fungiform papillae virtually disappear, so that instead of an epithelial surface with undulating waves, the dorsum of the tongue presents an entirely flattened appearance. Pegs of epithelium may project down into the underlying connective tissue. Such thinning of the epithelium may lead to secondary infection, so that a layer of leukocytes and fibrin is found adherent to ul-

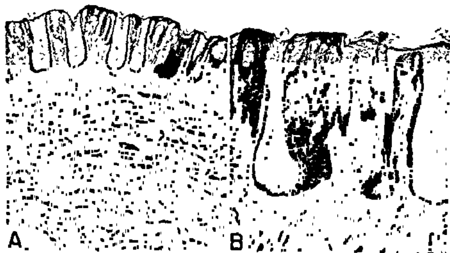
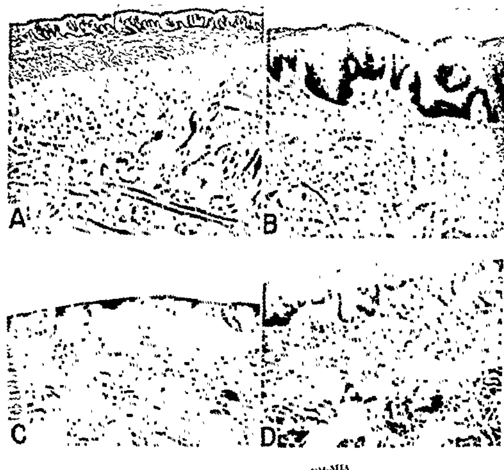


FIGURE 174. NORMAL TONGUE, HUMAN.

A (x 14), B (x 35) Epithelial lining of dorsum of tongue to show width of papillae.



...MIA

Tong ... , ... H 8391), *C* (x 14), and *D*. (x 35) (*J.H.H.* 7816) Epith ... of tongue from two individuals dying of pernicious anemia without specific therapy (before 1926). Compare with normal, Figure 174

cerated foci, in such areas mononuclear leukocytes may be encountered infiltrating the subepithelial connective tissue and muscle.

In association with the clinical evidence of intestinal involvement there may be ulcerative lesions of the colon; these are entirely non-specific in appearance.

Of the nervous tissues the spinal cord, particularly the thoracic portion, appears to bear the main brunt of the injury [1399, 1400, 1401] Lesions of lesser degree have been described in the peripheral nerves [1402, 1403] So, too, the brain may occasionally show morphologic alterations [1404, 1405] The earliest change in the cord develops as a focus of demyelinization, frequently about a blood vessel. Such foci are found first in the posterior columns and manifest themselves by the presence of neutral fat, lying free and in phagocytes.

The latter reaction, however, is not conspicuous Disappearance of the axoplasm accompanies the myelin degeneration Curiously enough, and unique for pernicious anemia, virtually no glial reaction appears As time goes on, the areas of degeneration in the white matter coalesce, so that more or less diffuse involvement of the posterior columns of Goll and Burdach is found. The absence of glial reaction gives the tissue a foamy appearance, the so-called, status spongiosus The pathologic process which, as already noted, tends to begin in the thoracic segments spreads upwards into the cervical cord and downwards into the lumbar area In time, foci of degeneration begin to appear in the lateral tracts When an entire cord is studied, the affernt tracts are found to be more involved in the cervical region while the efferent paths show the most change in the lumbar portion. Nowhere does the gray matter appear to be involved. The anterior and posterior roots show relatively little change, though they do not appear to have been ex-

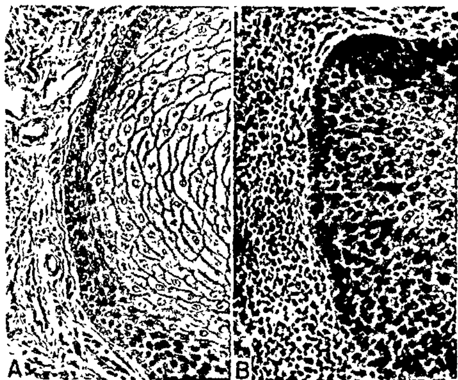


FIGURE 176 PERNICIOUS ANEMIA

Tongue Higher power of normal, *A* (x 265) and *B* (x 265) (J H H 7816), tissue from individual dying of pernicious anemia Note inflammatory reaction and difference in epithelial cells

A



FIGURE 178 PERNICIOUS ANEMIA

Spinal cord. *A* (J H.H 7816) Higher power of tracts shown in Figure 177 *B* Higher power of section shown in Figure 179 (J H H 8939)

amined as carefully as one would wish Nor are the dorsal root ganglion cells affected.

The peripheral nerves have been studied at autopsy[1402] and on biopsy.[1403] The myelin sheaths are said to be reduced in number, though there is no change in the axoplasm Counts have been necessary to bring out this change, an indication that it cannot be very marked The most peripheral portions of the nerve are said to be most severely affected Thus, though on clinical grounds the peripheral nerves have often been said to be involved, their damage may be difficult to demonstrate under the microscope.

That anatomical changes in the brain may occur in pernicious anemia has been recognized for some time[1404, 1405] Such foci begin and progress just as they do in the spinal cord, the white matter being affected, the gray matter spared Large foci of destruction of nerve fibers are sometimes found. Again, as in the spinal cord, there is virtually no glial reaction

←

FIGURE 177 PERNICIOUS ANEMIA

Spinal cord Sections from thoracic and lumbar cord from patient dying before 1926 to show widespread lesions in ascending and descending tracts (J H H 7816).

A

five membered rings consisting of dimethylbenzimidazole nucleotide and 1-amino-2 propanol.[1076] Structurally, it is the most complex of all the known vitamins

That vitamin B_{12} represents the extrinsic and erythrocyte maturation factors seems already established [1419, 1420] When large amounts of the crystalline material are administered orally to patients with pernicious anemia virtually all may be recovered in the stool. Nor is there any physiological evidence of absorption, i.e, a reticulocyte response. On the other hand, minute amounts (3 to 10 micrograms) are effective when administered parenterally. This difference in activity shows conclusively that the primary defect in the causation of the pernicious anemia syndrome is in the stomach The precise nature of this defect, that is the chemical substance or substances lacking, has not been as yet determined, though there is evidence that the intrinsic factor is a mucopolysaccharide.[1421]



FIGURE 179

Section of spinal cord from patient dying of pernicious anemia who had never received treatment (J H H 8939).

Optic atrophy has been observed, though not often, in pernicious anemia.[1406, 1407] It is important to realize, however, that on rare occasions disturbance in vision may be a presenting symptom.

Other less important findings at autopsy on untreated cases of pernicous anemia are pigmentation (hemosiderin) of the liver, spleen and bone marrow and fatty infiltration of the heart.

During the first twenty-five years of this century patients continued to die of pernicious anemia. To be sure remissions occurred in many, but once the diagnosis was made, the disease invariably progressed to a fatal termination The whole picture was dramatically changed in the spring of 1926 when Minot and Murphy announced the efficacy of liver in the treatment of forty-five cases of pernicious anemia.[1408] Particularly striking was the effect on the red blood cells as evidenced by a prompt rise in reticulocytes The initial report was further amplified.[1409] Nor did it take long for this important observation to be confirmed all over the world. Minot tells in his Nobel lecture[1410] that he was prompted to use liver because of its proven superiority in the studies of blood regeneration by Whipple[1411] and because of its effectiveness in the treatment of pellagra and sprue. Three years after the introduction of liver, Castle[1412] demonstrated the importance of the stomach and its secretions in the pathogenesis of pernicious anemia and clearly indicated that the syndrome must be regarded as a conditioned nutritional disease. When beef muscle was incubated with normal gastric juice and this mixture was administered to patients with pernicious anemia, a prompt remission occurred, as evidenced by a rise in reticulocytes. Shortly thereafter, Sturgis and Isaacs[1413] found that dessicated hog stomach was effective as a therapeutic agent in the presence of a normal diet.

Thus, in a few years' time these therapeutic studies led to a seemingly clearcut exposition of the pathogenesis of pernicious anemia: the absence of an *intrinsic factor* in gastric secretions led to an inability to form an *erythrocyte maturation factor* by combination with *extrinsic factor* furnished by the diet. This combination of *intrinsic* and *extrinsic factors*, i e., *maturation factor*, was stored in the liver. During the next twenty years attempts were made in many laboratories to identify the chemical nature of the *intrinsic* and *extrinsic factors*. Work was slow because of the lack of an experimental animal in which to study the disease Not until 1948 did the final solution unfold, based on the development of a suitable assay method—the use of the microorganism *lactobacillus lactis* Dorner.[1414] The isolation of a crystalline material (vitamin B_{12}) came soon after.[1415] The demonstration of its effectiveness in the treatment of pernicious anemia[1416] and in the arrest of spinal cord lesions[1417] followed immediately. The story was recently brought almost to its conclusion by the presentation of the probable structure of vitamin B_{12}, a cobalt containing porphyrin linked to

THE NON-ADDISONIAN MEGALOBLASTIC ANEMIAS

In addition to classical pernicious anemia, a group of blood dyscrasias characterized by macrocytic anemia with megaloblasts in the bone marrow have come to be recognized [1423, 1424] These anemias are associated with a number of conditions which may lead to disturbances in nutrition Folacin and/or vitamin B_{12} are usually effective in therapy. Table XIV is presented without any attempt at systematic classification, since our knowledge of the pathogenesis of each type of anemia is so vague at the present time. Measurements of vitamin B_{12} levels of serum and urinary excretion studies of folacin have begun to help clarify this group of anemias.[1424]

TABLE XIV

NON-ADDISONIAN MEGALOBLASTIC ANEMIAS

(1) Tapeworm Anemia
(2) Nutritional Macrocytic Anemia
(3) Anemia Associated with Intestinal Dysfunction
  (a) Tropical sprue
  (b) Idiopathic steatorrhea
  (c) Coeliac disease
  (d) *Intestinal strictures and anastomoses*
(4) Anemia Associated with Pregnancy and the Puerperium
(5) Megaloblastic Anemia of Infancy
(6) Macrocytic Anemia Associated with Cirrhosis of the Liver
(7) Megaloblastic Anemia Following Total Gastrectomy
(8) Miscellaneous Types

(1) TAPE WORM ANEMIA

The presence of the flatworm, *Diphyllobothrium latum*, in the upper intestinal tract leads to macrocytic anemia associated with megaloblastosis of the bone marrow. If extensive blood loss has been sustained as a result of the parasitic infestation, a hypochromic nomoblastic anemia may be encountered. The investigations of Bonsdorff [1425, 1426] appear to indicate that the worm produces a megaloblastic type of anemia in healthy individuals by interfering with the absorption of vitamin B_{12} from the intestinal tract The parasite takes up considerable amounts of vitamin B_{12}, thus depriving the host of this essential nutrient Neurological changes similar to those seen in pernicious anemia are found in some cases of this form of anemia.

(2) NUTRITIONAL MACROCYTIC ANEMIA

The studies of nutritional anemia in monkeys, which were initiated in India by Lucy Wills in the early 1930's, have been mentioned elsewhere

That vitamin B_{12} affects the erythropoeitic elements locally has been shown;[1422] for when small amounts of the vitamin are injected into the marrow space of the crest of the ilium of a patient with pernicious anemia in relapse, a local reversion of the cells from the megaloblastic to the normoblastic type takes place. On the other hand, free vitamin B_{12} does not appear to effect this reversion to normal in tissue cultures. "Bound" vitamin B_{12} is necessary. This discrepancy between the *in vivo* and *in vitro* effects is as yet not clear.[1420]

At the present writing vitamin B_{12} has been available for study for eight years; during that period a great deal has been learned about its metabolism in the normal subject and in the patient with pernicious anemia.[1418, 1420] Yet there are many perplexing questions related to pernicious anemia which have yet to be answered. Such are: the mechanism of combination of vitamin B_{12} with the *intrinsic factor*, the mechanism of absorption of this combination, the fate of ingested vitamin B_{12}, the significance of bound forms of the vitamin, its combination with other materials, such as bacteria in the gastrointestinal tract. Lastly, one must ask the question: can all the features of pernicious anemia be explained on a nutritional basis, i.e., the lack of vitamin B_{12}? Some feel the answer is "no" and postulate that a "toxic" factor is present, perhaps elaborated by bacteria in the gastrointestinal tract since megaloblastic anemia is associated with intestinal stenosis, blind loops of intestine, et cetera. It might be suggested that certain detoxifying mechanisms fail in the absence of vitamin B_{12}. Why is there no glial response in the spinal cord and brain? Now that the structural formula of vitamin B_{12} is known perhaps some of the answers to these questions will be forthcoming.

THE NON-ADDISONIAN MEGALOBLASTIC ANEMIAS

In addition to classical pernicious anemia, a group of blood dyscrasias characterized by macrocytic anemia with megaloblasts in the bone marrow have come to be recognized [1423, 1424] These anemias are associated with a number of conditions which may lead to disturbances in nutrition Folacin and/or vitamin B_{12} are usually effective in therapy. Table XIV is presented without any attempt at systematic classification, since our knowledge of the pathogenesis of each type of anemia is so vague at the present time Measurements of vitamin B_{12} levels of serum and urinary excretion studies of folacin have begun to help clarify this group of anemias [1494]

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(2) NUTRITIONAL MACROCYTIC ANEMIA

The studies of nutritional anemia in monkeys, which were initiated in India by Lucy Wills in the early 1930's, have been mentioned elsewhere

(page 271). These investigations indicated that in tropical and sub-tropical areas a macrocytic type of anemia with megaloblastic bone marrow may be encountered. Other forms of nutritional anemia may, of course, be found in these areas, particularly the microcytic type of iron deficiency. The anemia associated with the Kwashiorkor Syndrome was thought to be primarily macrocytic until it was realized that this feature must be ascribed to the presence of excessive numbers of reticulocytes which tend to be larger than normal, the bone marrow is normoblastic.[1232] The subject of nutritional macrocytic anemia is a confused one since deficiencies of other essential nutrients are almost invariably present. The real test is, of course, the therapeutic response to either folacin and/or vitamin B_{12}. The first successful demonstration of the effectiveness of folacin was carried out in patients with nutritional macrocytic anemia by Spies, *et al.*[1127] in 1945.

(3) ANEMIA ASSOCIATED WITH INTESTINAL DYSFUNCTION

(a) Tropical Sprue, (b) Idiopathic Steatorrhea, (c) Celiac Disease

The interrelationships of these three syndromes are not at all clear, though many workers[1435] feel that they represent variants of one fundamental disease rather than distinct entities The glossitis, flatulence, diarrhea without or with varying degrees of steatorrhea, and the anemia are familiar enough. The disease begins insidiously and may take years to develop. Success in therapy has been obtained by the administration of folacin and more recently of vitamin B_{12}. In a given case the response to either of these nutrients cannot be predicted with certainty, so that much remains to be learned of the pathogenesis of the various clinical entities which make up this group

The pathologic findings are not specific. The glossitis will resemble that found in other nutritional deficiency states. The stomach may show evidence of chronic inflammation. Recently, attention has been drawn to changes in the upper jejunum, which have been seen in biopsy specimens. Such alterations consist of atrophy of the lining epithelial cells with some cellular infiltration of the underlying submucosa[1535] The liver may show varying degrees of fatty infiltration.

(d) Intestinal Strictures and Anastomoses

That naturally occurring or surgically produced abnormalities of the intestinal tract can be associated with macrocytic, megaloblastic anemia was forcibly brought to our attention when we witnessed such an autopsy at the Johns Hopkins Hospital in 1938 Barker and Hummel[1428] have discussed the variable clinical picture in such cases, the symptoms and signs may be those occurring in the "sprue group." Again the effects of therapy,

aside from correcting the anatomical defect surgically, cannot be predicted with certainty.

At operation or at autopsy, strictures may be encountered. The etiology of these may not be evident, though tuberculosis has been encountered, so, too, benign or slowly growing malignant tumors, have been described. As to the surgically produced lesions one may expect to find most anything. In the case referred to above there had been three separate operations beginning with an appendectomy. The end result was an anastamosis between the jejunum and mid-colon producing two blind loops consisting of terminal ileum and proximal colon

(4) MEGALOBLASTIC ANEMIA OF PREGNANCY AND THE PUERPERIUM

This condition, sometimes called "pernicious anemia of pregnancy," appears to be a clear-cut clinical entity [1429, 1430] The onset is usually insidious: anemia is ordinarily not noted until the third trimester or even until the puerperium Glossitis and gastrointestinal disturbances may be marked. A macrocytic anemia is found The leukocyte count is variable. The bone marrow smear shows megaloblastic erythropoiesis, provided treatment has not been initiated Folacin is more effective therapeutically than is vitamin B_{12} or purified liver preparations The pathogenesis of this form of megaloblastic anemia is obscure, all causes have been advanced. intrinsic factor deficiency, defective diet, defective absorption or endocrine dysfunction [1430]

(5) MEGALOBLASTIC ANEMIA OF INFANCY

Cases of anemia with megaloblastic bone marrow in infants have been carefully studied and reported in recent years [1431] The disease develops insidiously with increasing anemia and anorexia The peripheral blood may or may not exhibit macrocytosis, hence bone marrow aspiration studies are extremely important The presence of scurvy in certain cases has raised the question that ascorbic acid deficiency might be playing a role. This possibility led May and his collaborators to study the effect of ascorbic acid deficiency in monkeys fed milk diets [1059] The conclusion reached is that ascorbic acid deficiency appears to increase the requirements for folacin but is not a direct cause of the anemia, which *responds to folacin therapy*

(6) MACROCYTIC ANEMIA ASSOCIATED WITH CIRRHOSIS OF THE LIVER

Some years ago Wintrobe [1432] called attention to the presence of macrocytic anemia in individuals with cirrhosis At autopsy foci of erythropoietic cells were found in the spleen Subsequent studies have corroborated the presence of macrocytic anemia in liver disease, the bone marrow may show

a megaloblastic picture. The pathogenesis of this type of anemia is not entirely clear. Whether the liver disease is the direct cause or whether the blood picture results from dietary faults are questions which remain to be answered.

(7) MEGALOBLASTIC ANEMIA AFTER TOTAL GASTRECTOMY

One would suppose that, since the gastric mucosa is the source of intrinsic factor, removal of the stomach would be followed by megaloblastic anemia This does occur, provided the individual lives long enough.[1490] The important point to remember is that most gastric resections are performed for carcinoma; the five year survival rate after such operations is abysmally low As one might expect, when anemia of a megaloblastic type does occur, vitamin B_{12} is effective.

(8) MISCELLANEOUS MEGALOBLASTIC ANEMIAS

Certain totally unrelated conditions may occasionally be associated with megaloblastic anemia.[1424] Such are leukemia, carcinomatous involvement of the bone marrow and endocrine disease, i.e., hypopituitarism.

All of the above types of non-addisonian megaloblastic anemia are of particular interest since their response to therapy is not uniform, that is in some the response to folacin is more satisfactory than to vitamin B_{12} and *vice versa* Such variations in response would indicate that the underlying deficiency in each must be worked out by careful studies of the metabolism of vitamin B_{12} and/or folacin. Studies employing vitamin B_{12}-containing radioactive cobalt or microbiological assays of vitamin B_{12} and folacin in blood and urine should provide an answer to these most interesting problems.

THE MALABSORPTION SYNDROME

In discussing the pathogenesis of deficiency disease on page 7, it was noted that interference with the absorption of essential nutrients was one of the important general factors giving rise to conditioned deficiency syndromes This has been brought out in the discussions of the pathogenesis of disease states such as the low-sodium syndrome, the hypokalemic syndrome, tetany, pellagra, blacktongue, kwashiorkor, rickets, tocopherol deficiency, and some of the megaloblastic anemias. Diarrhea, with or without disturbance in the metabolism of lipids and other nutrients, may be an inciting or contributory factor in any one of these syndromes On the other hand a group of disease entities has been recognized for over fifty years and has achieved the status of syndromes in their own right In the continuing state of our ignorance it appears best to designate them under a single term, *the malabsorption syndrome.*[1435] The syndromes to which we refer are tropical and non-tropical sprue, celiac disease and idiopathic steatorrhea.[1436, 1437] In all of them gastrointestinal disturbance dominates the clinical picture Since the pathologic findings in the intestinal tract are meagre, and our understanding of the pathogenesis leaves much to be desired, they have usually been designated as idiopathic Diseases, in which hepatic, pancreatic or intestinal lesions are present, must be excluded.

Clinically these diseases occur at all ages. Celiac disease is the term given to the syndrome occurring in infancy and childhood, while nontropical sprue and idiopathic steatorrhea are synonymous terms referring to the adult syndrome. They are characterized by growth failure in children, marked weight loss; severe diarrhea with gross or chemical evidence of excessive lipid in the stool, disturbed intestinal motility by x-ray, a flat glucose tolerance curve, anemia which is frequently macrocytic in character; hypoproteinemia with edema; hypocalcemia with tetany, rickets or osteomalacia, bleeding tendencies and electrolyte disturbances

The place of tropical sprue in the clinical picture is less clear Originally this entity was described before nontropical sprue was recognized Today there are few if any differences save that the syndrome occurring in the tropics tends to be more severe and to respond more satisfactorily to therapy

The conditioned deficiencies which may be observed in the malabsorption syndrome are often severe As was already mentioned steatorrhea is marked This is found in the presence of pancreatic enzymes in the intestinal contents As a result of poor absorption of lipids, there may be evidences of deficiencies of the fat-soluble vitamins, particularly A, D, and K. Hence, the disturbances which characterize deficiencies of those nutrients may be pres-

ent. Mineral absorption is diminished: sodium, potassium, calcium, phosphorus and iron have all been shown to be affected. Nitrogen balance is usually negative. So, too, glucose absorption is depressed. Fructose uptake appears to be unaffected.

As has already been noted, other causes of diarrhea or steatorrhea must be excluded. These include hepatic or pancreatic deficiency, intrinsic disease of the stomach, intestine and colon, disturbances in lymphatics and the diarrheas caused by animate agents. Pathological examination reveals very little save atrophy of the cells of the intestinal mucosa. It is apparent to most gastroenterologists that this group of diseases may have no single etiology. Several causes are currently in favor. The first relates the disease to a familial or constitutional background[1435, 1437] If the siblings of severely affected children are examined, evidences of disease may be found. So, too, the disease may be seen in several members of a family of adults. The second clue concerning the etiology has assumed prominence since 1950, this is based on observations that remissions and improved fat absorption could be attained if wheat and rye gluten were removed from the diet. These have been corroborated in several clinics where children[1289, 1438] and adults[1439] have been observed. Such studies indicate that the basic disturbance may be looked upon as due to an effect of the glutamin bound to gliadin of the wheat or rye gluten. The underlying biochemical disturbance has not yet been elucidated. Cortisone therapy is also effective; the basis for this is entirely obscure.[1440]

Finally, the role of specific nutrients themselves in causing certain portions of these syndromes must be considered. Certain manifestations such as megaloblastic anemia are clearly related to deficiency of folic acid or vitamin B_{12}. At least these nutrients lead to a prompt repair of the hematological picture. Hence, it is difficult to decide which comes first and at the moment one must wait until more is known of the pathogenesis of the entire group.

DENTAL CARIES

During the past decade, dental caries, which has been called America's most expensive disease, has received the attention it deserved. Among the many factors which may affect the integrity of the tooth and alter its susceptibility to decay, its nutrition is of importance, first during the formative stage, then while erupting and maturing, and finally for the rest of its adult life Hence, a brief discussion of the dental caries problem is warranted, although we cannot review in any detail the many contributions which have appeared during the past ten years or more. The AAAS Symposium, "Advances in Experimental Caries Research," is an excellent introduction to the whole problem.[1458]

What is dental caries and how does it develop? The lesion can be defined in terms of its evolution [1459, 1460] The initial change, whether in man or the experimental animal, begins on the surface of the tooth, hence the earliest alterations to be found in the enamel are of importance Here histochemical studies and observations utilizing the electron microscope have been rewarding.[1461] The first change is the formation of a tiny plaque on the surface of the enamel This plaque is composed of bacteria and debris If it is gently removed small pits and accentuations of the ends of the enamel prisms can be demonstrated. As this alteration progresses the enamel interprismatic matrix material begins to disappear. At this stage one can see a lesion with the light microscope, such consists of a small focus of destruction of the enamel which involves partial or complete removal of the inorganic materials as well as the organic matrix. Large numbers of bacteria of various types are found in such foci. They grow down into the dental canals and continue on to involve the side branches of these structures In time, as more enamel is destroyed, the dentino-enamel junction is breached Here the process continues, destruction of the inorganic and organic constituents of the dentine occurs Finally, when the dentine is destroyed the pulp cavity naturally becomes involved

The location of the lesions just described varies, at least in the experimental animal, with respect to type of diet, particle size, carbohydrate content, et cetera However, the enamel defects begin most frequently in fissures They are remarkably symmetrical Sometimes one can observe grossly the lesions spreading and see the tooth substance of the cuspal walls beginning to break down while the lesion spreads farther Special studies, such as examination of the jaws after removal and drying, show up the carious lesions as small foci of brownish discoloration on the enamel surface These areas are softer than the surrounding enamel when they are tested

ent. Mineral absorption is diminished: sodium, potassium, calcium, phosphorus and iron have all been shown to be affected. Nitrogen balance is usually negative. So, too, glucose absorption is depressed. Fructose uptake appears to be unaffected.

As has already been noted, other causes of diarrhea or steatorrhea must be excluded. These include hepatic or pancreatic deficiency, intrinsic disease of the stomach, intestine and colon, disturbances in lymphatics and the diarrheas caused by animate agents. Pathological examination reveals very little save atrophy of the cells of the intestinal mucosa. It is apparent to most gastroenterologists that this group of diseases may have no single etiology. Several causes are currently in favor. The first relates the disease to a familial or constitutional background[1435, 1437] If the siblings of severely affected children are examined, evidences of disease may be found. So, too, the disease may be seen in several members of a family of adults. The second clue concerning the etiology has assumed prominence since 1950, this is based on observations that remissions and improved fat absorption could be attained if wheat and rye gluten were removed from the diet. These have been corroborated in several clinics where children[1289, 1438] and adults[1439] have been observed. Such studies indicate that the basic disturbance may be looked upon as due to an effect of the glutamin bound to gliadin of the wheat or rye gluten. The underlying biochemical disturbance has not yet been elucidated. Cortisone therapy is also effective; the basis for this is entirely obscure.[1440]

Finally, the role of specific nutrients themselves in causing certain portions of these syndromes must be considered. Certain manifestations such as megaloblastic anemia are clearly related to deficiency of folic acid or vitamin B_{12}. At least these nutrients lead to a prompt repair of the hematological picture. Hence, it is difficult to decide which comes first and at the moment one must wait until more is known of the pathogenesis of the entire group.

examined, the odontoblasts, as well as their respective matrices of dentine and enamel, were normal, save for hemorrhage and cysts in the enamel organ of the younger child. It is likely that growth of the human tooth is too slow for this structure to exhibit morphological changes.

It will be recalled that vitamin A deficiency leads to disturbances in enamel formation (page 137). No experimental evidence is available that vitamin A deficiency affects the prevalence of dental caries in experimental animals

On the other hand the early observations of Mellanby on tooth changes in rachitic dogs[529] and the caries observed by McCollum[1463] in rats on calcium-deficient diets directly implicated vitamin D, calcium, and phosphorus in the formation of poorly mineralized enamel matrix and dentine The role of previous rachitic alterations in susceptibility to tooth decay has been much debated. There is some evidence that a positive relationship exists.[1464]

The possible roles of protein and magnesium deficiences (pages 86 and 40) in affecting the development of dental caries have not been studied to any extent, this problem must be investigated in the future.

Of the greatest importance among the essential nutrients, whose specific relation to the teeth places it in the indispensable class, is fluorine (page 81). This is particularly true as far as human disease is concerned and is, of course, the foundation of the fluoridation program It is not necessary to discuss this subject here save to reiterate that fluoride must be administered during the formative and eruptive stages if it is to be effective

As yet, unidentified factors, probably nutritional, have been found to affect caries susceptibility When purified diets, complete in all known essentials, are fed to rodents (rats, hamsters and mice) caries incidence is increased over that observed when a non-purified stock diet is employed.[1465] Hence, there is more than suggestive evidence that other factors are necessary than those ordinarily considered to be indispensable Protection against dental caries thus becomes another index of indispensability.

After the teeth have been formed and begin to erupt, a certain degree of maturation occurs until these structures reach full development and the third stage, that of maintenance, is reached There is evidence from studies with radioactive calcium, phosphorus, and iodine that exchanges are higher between the external or internal environment and the inorganic components of the enamel and dentine in a young than in an older tooth[1466] During these periods the profound effects of the type of diet, the saliva and bacterial flora begin to make their contributions to caries production

The importance of the saliva in maintaining the integrity of the teeth is well-known For instance, caries tends to increase in the human when disturbances associated with absence of salivary secretions are present. Hence

with a sharp instrument. Dullness of the surface and changes in translucency are then apparent

In summary, the development of the carious lesion may be delineated as follows. plaque formation, destruction of superficial enamel, enamel penetration, dentine invasion, and pulp involvement.

In considering the pathogenesis of these changes two areas must be examined. (1) the basic structure of the tooth itself and how it may be modified, and (2) the external or oral environment of the tooth, with particular respect to saliva, foodstuffs and bacteria.

It is obvious that there is not one but many causes of dental caries. Such factors can be divided, as is usual in any discussion of the pathogenesis of disease, into three categories: (1) Genetic, (2) Internal environment, particularly during growth and development, and (3) External or oral environment. Some important aspects of these factors may now briefly be mentioned.

The first question which comes to mind is, "Do genetic factors play a role in caries susceptible organisms?" The answer is "yes."[1462] Strains of rats have been bred, some are extremely caries-resistant, while in others on similar diets tooth destruction may be excessive. Genetic factors appear to be related to differences in sex, to the age at which lesions develop, to the ease with which enamel fractures, and to the presence and size of fissures, all of which affect the development of carious lesions.

The structure of the developing tooth can be modified *in utero* by a number of nutritional factors. A similar situation may operate at birth, since the teeth, particularly the permanent ones of man, are still developing in their bony environment and erupting for many years. Hence, the adequacy of essential nutrients, whether they be inorganic elements, amino acids, or vitamins, are undeniably important. The role of such essential nutrients on the integrity of the tooth has been noted in a number of places in this monograph. minerals, such as magnesium (page 40) or fluorine (page 81); vitamin D, calcium and phosphorus (page 156); protein depletion (page 86), vitamin A deficiency (page 187); and ascorbic acid deficiency (page 191). The relation of each of these materials to the dental caries problem must be considered during the three eras in the life-history of a single tooth, i.e., during development, maturation, and maintenance.

During the first or developmental period, the effect of vitamin C deficiency on dentine formation makes itself felt in the experimental animal (page 191). Alterations in the ameloblasts and enamel are secondary, probably the result of lack of support by the underlying dentine. No evidence to indicate a relationship of vitamin C deficiency to dental caries has been presented. When the tooth germs of two infants, aged eight and eleven months, respectively, whose bones exhibited classic signs of scurvy, were

NUTRITIONAL MELALGIA

(THE BURNING FEET SYNDROME)

One of the most interesting and as yet unexplained manifestations of nutritional deficiency is the so-called "burning feet syndrome" or, a more appropriately suggested name, "nutritional melalgia."[1455] This syndrome has been described in civilian populations subsisting on parboiled rice but assumed prominence as a major malady among American Forces and those of other nations interned in Japanese prisoner of war camps.

Historically, shooting pains or burning sensations in the feet have long been recognized in tropical areas. The following description is taken from the reports of Cruickshank[1457] and Glusman[1455] The onset is gradual, the first subjective complaints are numbness or pins and needles and tinglings of the toes and feet After a few weeks the parasthesias changes to burning pains in the toes and soles of the feet This pain is usually of a dull boring type. In more severe cases periodic shooting pains seem to originate from between the first and second toes and extend along the dorsum of the foot and up the legs in a severe shock-like fashion The pain is worse at night. A few individuals also have similar though not such severe pains in the palms of the hands Sweating may accompany the pains Relief is obtained by placing the extremities in cold water, on the cold ground, or in snow. Even when the disturbance becomes chronic, the reflexes are preserved and no pyramidal tract involvement nor any spasticity is evident The affected individuals have a peculiar gait, walking cautiously and gingerly with a flat-footed base Careful examination of the skin may reveal patches of dilated capillaries Loss of posterior tibial and dorsal pedis arterial pulsations have been observed, followed by gangrene. Many of the prisoner of war subjects had stomatitis and/or scrotal dermatitis In some of the cases observed by Cruickshank[1457] there was spastic paraplegia.

The pathogenesis of this syndrome is not clear, i.e., whether nervous or circulatory in origin Gopalan[1456] found little or no improvement with thiamine, riboflavin or nicotinic acid However, he reports dramatic improvement following the administration of calcium pantothenate Confirmation of this most interesting and important observation has been provided, in part at least, by the development of painful extremities in subjects made deficient in pantothenic acid by the antagonist omega–methyl-pantothenic acid[902] On the other hand, polyneuritis has also been observed in a patient in whom pyridoxine deficiency had been produced by the administration of the antagonist, desoxypyridoxine.[948]

studies of animals, whose salivary glands have been removed, are of interest.[1467] Salivary adenectomy in white rats, hamsters and cotton rats leads to marked increases in the incidence of caries. Unilateral removal leads to an increased incidence on the operated side. The secretions of the parotid and submaxillary glands appear to be of greater importance than those of the sublingual or extraorbital lacrimal glands. When the oral washings of adenectomized animals are compared with those of controls, a larger amount of protein and alpha amino acid nitrogen is found in the former groups Flow of saliva thus appears to clear the mouth of such materials. Doubtless other *factors, such as enzymes, inorganic components, et cetera, in saliva* seriously affect the integrity of teeth; these remain to be more fully elucidated.

It will be recalled that the initial change observed under the electron microscope is the formation of bacterial plaques. The role of bacteria in the development of caries in the experimental animal has been studied in a variety of ways. For instance, when germ-free animals are utilized no caries develops. Hence the conclusion has been drawn that dental caries in the rat is not possible in the absence of microorganisms [1468] Antibiotics which modify the oral flora will in turn decrease the incidence of caries. The synergistic actions of food and bacteria are also of importance. When animals are fed by stomach tube, the incidence of caries is diminished or prevented.[1469] Moreover, the actions of bacterial enzymes on food, particularly carbohydrate, in the production of lactic and other acids, would appear to be of importance in leading to localized foci of destruction of the inorganic components of enamel.[1470] Caries is virtually nonexistent in diets deficient in carbohydrate.[1471] Changes in pH may affect the integrity of dentine after *the enamel break-through has been consummated*

The type of food, particularly with respect to particle size has been shown to be important in caries development. Large insoluble masses of debris, as well as particles which mechanically mar the enamel, may be particularly detrimental.

In recent years the caries problem has obviously become more complex yet the realization of its complex nature has served to delineate more clearly pathways *for future attack. One of these deals with deficiency states* How important this phase is remains to be determined

MISCELLANEOUS SYNDROMES

When one surveys from a distance the literature of the past quarter century, which is not too difficult an operation if one consults the indispensable *Nutrition Abstracts and Reviews*, he is impressed by the large number of natural disease syndromes which have been ascribed to faulty nutrition. This is particularly true of the diseases in farm animals which have a most picturesque terminology, which someone should take the time to assemble in one place. Another group is that which was described among the human occupants of concentration and prisoner of war camps during World War II.

Such a survey is rather exasperating to one who has been brought up to try to obtain some understanding of the pathogenesis of disease by its study after death. In most instances of the diseases which one can read about this has not been done. That is, of course, why they must be lumped into a miscellaneous group. Sometimes examinations, during life or postmortem, could not be accomplished This is particularly true of the human syndromes which were observed in World War II. Fortunately, too, such diseases were not necessarily fatal. As far as the naturally occurring diseases in farm animals are concerned, advances are being made today as a result of the careful investigations being carried out at Experimental Stations and Institutes throughout the world

For those who may wish to have an introduction to the literature dealing with the neurological manifestations of deficiency disease, to parotid enlargement, to effects on the endocrine systems, et cetera, some references which we have found interesting are appended [1457, 1490, 1497, 1498, 1499, 1500, 1501, 1502, 1503, 1504] Contributions such as these, though they may be interesting, are disquieting, for they illustrate *par excellence* how little we know concerning the pathogenesis of so many of the abnormal states which we call deficiency disease.

Part VIII

Pathologic Physiology and Anatomy of Specific Tissues—a Recapitulation and Comparison

INTRODUCTION

Many of the preceding sections of this monograph have been concerned with changes resulting from deficiencies of single essential nutrients Most of these deficient states have been studied under controlled conditions and hence have been clearly defined Disease syndromes, particularly those which are observed under natural conditions in the human and which result from deficiencies of multiple nutrients, have also been described. In all of these deficient states a large number of physiological and anatomical alterations have been cited and classified, whenever possible, on an etiological basis At this point it may be of interest to summarize or recapitulate these alterations on a morphological basis. We beg pardon for such an approach to the nosography of disease, for it is one which to our mind has been outdated for some time Here, however, it enables us to compare and to amplify some of the data which have been reported in the preceding pages

Summaries of this section are presented in a series of tables. Virtually every tissue in the organism, which has been at all carefully studied, has been found to be the site of physiologic or anatomic disturbances as a result of exogenous or endogenous deficiency states It will be necessary in the case of some instances of naturally occuring disease to discuss them separately from those which may be studied in the laboratory

EPITHELIAL TISSUES

SKIN

The skin is composed of three parts (a) epidermis, (b) corium or dermis, and (c) subcutaneous tissue The epidermis consists of a superficial epithelial cell layer and two types of appendages. (a) sweat and sebaceous glands, and (b) hair follicles with hair

The skin has been studied extensively in experimental deficiency states and, of course, provides an excellent index of the state of nutrition under natural conditions. Lesions which have been reported thus far in the epidermis and its appendages, particularly in the rat, furnish examples of the many divergent alterations which may be found. All of the common changes seen in naturally occurring dermatologic disease may be produced in the experimental animal Such alterations as: (a) hyperkeratosis (increase in

PART VIII

PATHOLOGIC PHYSIOLOGY AND ANATOMY OF SPECIFIC TISSUES—A RECAPITULATION AND COMPARISON

TABLE XV

PATHOLOGIC ALTERATIONS IN SKIN [illegible] AS A RESULT OF EXPERIMENTAL [illegible]

1 *Epidermis*

(a) Atrophy: Calorie, protein, riboflavin [illegible] pyridoxine, biotin, vita-
(b) Hyperkeratosis: Magnesium, zinc [illegible]
min A (if previously atrophic), essential [illegible]
(c) Parakeratosis: Zinc [illegible] fatty acids
(d) Acanthosis: Zinc, pantothenic acid [illegible] acids
(e) Pigment: Copper, cystine, pantothenic acid [illegible]

2 *Hair Follicles* (Alopecia)

Zinc, biotin, riboflavin

3 *Sebaceous glands*

Zinc, biotin, riboflavin

4 *Corium*

Riboflavin (atrophy), vitamin C (failure to repair

5 *Blood Vessels*

Magnesium, pyridoxine, ascorbic acid

The large amounts of keratin which are lost from the human each day indicate the great proliferative activity of the epidermal cells

All of the chemical transformations concerned with the phenomenon of keratinization are not known One may observe the appearance and growth of the sulfur containing keratohyaline granules in epithelial cells The production of excess keratin or interference with its normal maturation processes obviously indicates some disturbance in the metabolism of this scleroprotein and the epithelial cells which form it Exactly how the nutrients listed in Table XV fit into an orderly scheme of these processes await a complete biochemical understanding of all the steps in keratin production. Only then will the significance of the hyperkeratosis seen in starvation or semi-starvation in man, in the pellagra syndrome or in kwashiorkor be understood Thus knowledge, too, must precede our interpretation of the hyperkeratosis seen in scurvy and experimental ascorbic acid deficiency, in the experimental human syndrome produced by vitamin B_6 deficiency and in the lesions which develop on a tryptophan-niacin low diet.

Pigment: Much has been learned in recent years concerning the biochemistry of melanin formation and its relation to tyrosine metabolism [1508] Currently it is believed that the oxidation of tyrosine to melanin is catalyzed by a copper containing enzyme, tyrosinase and that this reaction is activated by certain dehydroxyphenyl compounds such as *dopa* Other agents may inhibit the reaction The cell responsible for the pigmentation of skin and hair is the melanoblast Most of the studies dealing with alterations in pigment production by deficiency state have been restricted to alterations

keratinized fibers on the surface); (b) parakeratosis (incomplete keratinization), (c) acanthosis (increase in thickness of middle skin layers); (d) metaplasia (change from one cell type to another); (e) seborrhea (excessive activity of sebaceous glands with collection of their secretion on the surface), (f) alopecia (loss of hair), and (g) pigment changes have been described. Under the microscope alterations observed in the experimental animal may not be too specific. This is also true of naturally occurring dermatological disease in man. However, when one also takes into consideration the individual peculiarities of distribution over the body surface and the sequences in progression, rather specific syndromes may be defined, which Sullivan and his co-workers have done much to develop. Our knowledge of skin lesions in species other than the rat is fragmentary; hence, much needs to be done. This is especially true for skin lesions which are associated with deficiency states in the human.

So far as it has been studied, the skin appears to be no different from other tissues in its general metabolic processes. Hence, it is reasonable to conclude that the energy metabolism of the skin is derived from phosphorylative glycolysis, the citric acid cycle, and dehydrogenase activity through to the cytochrome system. One would therefore suppose that certain inorganic elements such as magnesium, zinc, manganese, iron and copper might be necessary for the activity or structure of its enzymes. Moreover, certain vitamins such as nicotinic acid amide, riboflavin, thiamine, lipoic acid, and pantothenic acid would be necessary to furnish DPN, TPN, flavoproteins, co-carboxylase, et cetera. Furthermore, since the metabolism of the epidermis is so concerned with protein synthesis, particularly keratin production, pyridoxine might also be of importance. All of these suppositions turn out to be the case, though we do not know how each of the essential nutrients fits as an individual link in the entire chain of events.

In order to discuss changes in the skin we shall take up various anatomical parts and indicate some of the alterations which have been encountered. These are summarized in Table XV.

Atrophy of the epidermis and its appendages is seen as a non-specific response to malnutrition, i.e., as a result of caloric restriction (page 12), protein deprivation (page 86) and multiple vitamin deficiencies (page 15). This is, of course, responsible for much of the change which has been described in instances of human starvation.

Hyperkeratosis, accompanied by *acanthosis* and less frequently by *parakeratosis*, is the most commonly encountered change in the epidermis, particularly in naturally occurring syndromes in the human; though as will be noted in Table XV many single deficiency states are characterized by hyperkeratosis. Under normal circumstances the epithelial cells undergo certain structural changes as they are transformed to keratin and shed.

TABLE XVI

EPITHELIAL LESIONS IN THE HUMAN ASSOCIATED WITH EXPERIMENTAL OR NATURALLY OCCURRING DEFICIENCY STATES

Erythematous or Scaling Dermatitis Tryptophan-niacin, pyridoxine, biotin, pellagra, kwashiorkor
Seborrhea: Riboflavin, tryptophan-niacin, pyridoxine, pellagra
Hyperkeratosis: Ascorbic acid, scurvy
Scrotal Dermatitis Riboflavin, pellagra
Vaginitis Tryptophan-niacin, pellagra
Cheilosis Riboflavin, tryptophan-niacin, pyridoxine, pellagra, kwashiorkor
Glossitis Iron, tryptophan-niacin, pyridoxine, folacin, vitamin B_{12}, biotin, pellagra, pernicious anemia, non-Addisonian megaloblastic anemias

EYE AND PARAOCULAR TISSUES

Cornea: One of the earliest and most dramatic effects of nutritional deficiency to be described was vascularization of the cornea, this was attributed to be a specific effect of a lack of riboflavin. Since that time, ingrowth of capillaries into the normal avascular cornea has become commonplace and has been observed in deficiencies of elements (sodium, zinc), amino acids and a number of vitamins Hence, corneal vascularization has come to be regarded as one of the more common examples of non-specific change resulting from dietary deficiency. What this means is not entirely clear The metabolism of the cornea has been and continues to be studied intensively It is a unique tissue since it contains no blood vessels and must obtain its nutrients by diffusion from the pericorneal vessels, from the tears, and from the aqueous The vascular change must be interpreted as a response to injury, just as blood vessels may be made to grow into the cornea if the tissue is traumatized in a variety of ways Not enough attention has been given to the intimate alterations which are present in the epithelial cells, in their regenerative activity which, of course, is great, and in the physical characteristics of the corneal collagenous fibers and associated mucopoly-

TABLE XVII

PATHOLOGIC ALTERATIONS IN THE EYE AND PARAOCULAR GLANDS AS A RESULT OF SINGLE DEFICIENCY STATES

(1) *Cornea.* Sodium, zinc, essential amino acids many vitamins
(2) *Conjunctiva* Sodium, vitamin A
(3) *Lens* Calcium, essential amino acids, riboflavin
(4) *Intraocular structures* Ascorbic acid (aqueous)
Vitamin A (retina)
(5) *Paraocular glands*
(a) Lacrimal. sodium, vitamin A
(b) Tarsal sodium
(c) Hardenan. pantothenic acid

in the color of hair. Graying or achromotrichia has been observed on numerous occasions. Copper-deficient animals, as might be expected show loss of hair color. So, too, do lysine-deficient rats. Two vitamins, pantothenic acid and para-aminobenzoic acid, exhibit an effect on hair pigmentation.

Alopecia: A most obvious manifestation of deficiency disease, one which has been recognized for some time, is loss of hair. Hair, which is a complex keratin-containing material is formed by the epithelial cells which make up the hair follicles. Hence, any alteration in hair or wool of animals or the hair of man must be related to the integrity of the cells of the follicles. Much is now known of the various biochemical and histochemical transformations which take place as the cells of the follicles grow, differentiate and form the first hairs [2369] The growth of hair in certain species has cyclic activity. Three main stages have been delineated: (1) Anagen or growing period, (2) Catagen or transition from growing to resting period, and (3) Telogen or resting stage. The anagen stage has been divided into as many as six sub-stages This cyclic activity of hair will, of course, affect the development of alterations produced by deficiency states depending upon when the subject becomes deficient with respect to the cycle.

It would not appear necessary to indicate the various deficient states which are associated with alopecia. These are listed in Table XV.

Sebaceous Glands: The sebaceous glands have not been carefully studied in experimental animals. Curious alterations consisting of increase in size of the cells have been described in zinc deficiency (page 71), while necrosis has been observed in riboflavin-deficient rats (page 210).

Corium: Whenever there is failure to grow the collagenous fibers of the corium may be atrophic This is most distinctly seen as a result of riboflavin deficiency (page 210). Perhaps the most dramatic change is encountered in ascorbic acid deficiency. Here the fibroblasts are unable to promote the formation of new collagenous fibers (page 188).

Blood Vessels: The physiological response of the blood vessels has been imperfectly studied in deficiency states. It would appear from the few isolated observations which have been published that here would be a fruitful field for research. For instance, the dilatation which is seen in magnesium or pyridoxine-deficient rats needs to be further investigated, particularly with respect to their response to pharmacologic agents such as those which have been used to explore the vascular defect in vitamin C deficiency.

•

Deficiency of a number of essential nutrients may lead to alterations in the skin and its appendages in the human. These changes are summarized in Table XVI.

Lens: Cataract is also commonly associated with deficiencies and it is likely that this structure may have been overlooked in experiments dealing with nutritional deficiencies in the past. The avascular lens would seem to respond to somewhat the same deficiencies as does the cornea. The metabolic needs of the lens are related to its ability to maintain its hydration and hence its transparency, so that any deviations in the former may be expected to lead to cataracts.

Intraocular Structures: Ascorbic acid is necessary for the secretion of the aqueous. Differences in tension have not been described as a result of other deficient states. Both vitamin A and riboflavin are found in the retina. Changes have only been noted as a result of deficiency of the former. These are hemeralopia and changes in the ganglion cells. The latter may be due to pressure effects on the optic nerve by bony overgrowth or increased intracranial pressure.

Lacrimal Glands: The most extensive changes occur in the ducts of the periocular glands as a result of vitamin A deficiency. Hyperkeratosis leads to obstruction of the ducts which results in atrophy of the glandular epithelium and absence of secretion. The tarsal glands of sodium-depleted rats are obstructed, apparently by a caking of secretion along the lid margins. In rats deficient in pantothenic acid the Harderian gland secretes an excessive amount of pigment which has been demonstrated to be corproporphyrin.

THE GASTROINTESTINAL TRACT

Lips: Labial lesions have not been prominent in single deficiency states which have been studied in experimental animals. However, in the human cheilosis has been a common sign of nutritional deficiency.[1510] Studies of single deficiency states in human subjects have revealed that several essentials may be implicated. These are riboflavin (page 217), tryptophan-niacin (page 220), and pyridoxine (page 248).

Tongue: The prominence of tongue lesions in human deficiency states such as pellagra and pernicious anemia has focused attention on this struc-

TABLE XVIII

PATHOLOGIC ALTERATIONS IN THE GASTROINTESTINAL TRACT AS A RESULT OF SINGLE DEFICIENCY STATES

(1) *Lips* Riboflavin, tryptophan-niacin, pyridoxine
(2) *Tongue* Iron, zinc, riboflavin, tryptophan-niacin, pyridoxine, biotin, pantothenic acid, *folic* acid and vitamin B_{12}
(3) *Salivary Gland* Vitamin A
(4) *Esophagus* Zinc
(5) *Stomach* Calcium
(6) *Small Intestine* Potassium (*motility*)
(7) *Large Intestine* Pantothenic acid

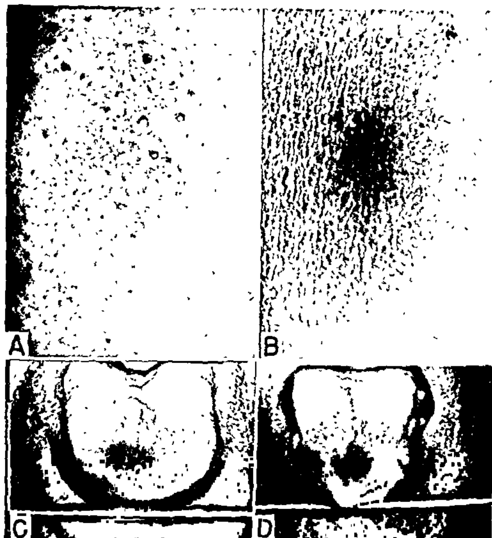


FIGURE 180.

A. Follicular hyperkeratosis, nonspecific. *B.* Hyperkeratosis, nonspecific. *C.* Tongue, prominence of follicles. *D.* Tongue, atrophy of papillae. These four photographs indicate certain lesions which are commonly seen in deficiency states (Courtesy of Dr A Schaeffer)

saccharide It is likely that in some instances the vascular change may be secondary to gross epithelial alterations, such as occurs in vitamin A deficiency, while in others the alteration may be a subtle biochemical lesion.

Conjunctiva: Here alterations such as those associated with vitamin A deficiency are clear enough, though how much of a role disturbances in the secretion of the lacrimal and other glands play is difficult to say at this time.

LIVER

As might be expected, the liver has been studied most extensively from the biochemical standpoint. Much is known, too, of the morphologic alterations which result from deficiency states. In recent years a good deal of attention has been given to two profound alterations in the liver which result from deficiencies of essential nutrients These are the acute necrosis syndrome and the fatty infiltration-cirrhosis complex. The main similarities and differences and the nutrients which pertain thereto have been summarized in Table XIX.

TABLE XIX

DIETARY FACTORS ASSOCIATED WITH LIVER INJURY

	Necrosis	*Fatty Liver—Cirrhosis*
Cystine	+	−
Methionine	+	+
Protein	+	+
Choline	0, −	+
Tocopherol	+	0
Factor 3 (selenium)	+	0
Threonine		+
Lysine		+
Tryptophan		+
Essential fatty acids		+
Lipid	0, −	−
Vitamin B_{12}		+
Riboflavin		+
Pyridoxine		+
Pantothenic acid		+
Antibiotics		+

+ = Beneficial
− = Harmful
0 = No effect

Since the delineation of the acute necrosis syndrome in 1935 our understanding of it has undergone a slow evolution so that now three and perhaps four factors may be indicted These are cystine (page 98), alpha-tocopherol (page 169) and Factor 3 (page 101), in which selenium has recently been postulated to be the important component. In addition, the possible role of extraneous positive factors, particularly in the form of bacterial products absorbed from the gastrointestinal tract must not be lost sight of (page 257). Cystine deficiency may be modified, depending on how much methionine and protein are included in the diet. The destruction of alpha-tocopherol by unsaturated fatty acids, such as are found in cod liver oil, leads to more extensive necrosis. The role of excess yeast and the protective effects of selenium have just been reported. Developments here will be awaited with great interest.

ture as an index of nutrition for some time. The lack of specificity of lingual changes has become only too apparent in recent years. In the human subject tongue lesions may follow deficiencies of iron (page 299), riboflavin (page 218), tryptophan-niacin (page 220), pyridoxine (page 248), biotin (page 266), folic acid (page 436), and vitamin B_{12} (page 425), (see Table XVI).

The tongue of dogs deficient in various members of the vitamin B complex has been specifically studied by Afonsky.[1512] Deficiencies of niacin, riboflavin, pyridoxine, folic acid and pantothenic acid were found to be associated with lingual changes. These alterations varied according to the type, duration and severity of the deficient state. As might have been expected the microscopic changes were not pathognomonic. The changes common to all were papillary atrophy and changes in the epithelial cells The lesions did not appear to be related to alterations in other areas of the gastrointestinal tract.

The explanation for the prominent involvement of the tongue in human and canine deficiencies is not clear, though doubtless the extraordinary normal regenerative activity must be of significance.

Salivary Glands: These structures are involved as a result of vitamin A deficiency (page 126). The role of dietary deficiency in cases of parotid enlargement which have been observed in malnourished humans is not clear.[1193, 1497]

Esophagus: A most unique alteration has been reported in the esophagus of zinc-deficient rats in which there is thickening of the epithelium due to the presence of partially keratinized cell layers (page 72). Similar changes have not been described in other deficiency syndromes, though it is unusual to find experiments in which this structure is said to have been examined.

Stomach: Ulcers of the stomach have been reported to result from a number of deficiencies of necessary nutrients, in many instances such diets have lacked other essentials so that the specificity of the lesions must be questioned. Calcium deficient rations, however, lead to ulceration of the gastric antrum in rats and to gastric lesions in dogs (page 48).

Intestine: The motility of the small intestine appears to be affected in potassium-deficient animals (page 25). Epithelial changes occur in a nonspecific alteration in response to caloric restriction and a number of vitamins.[11] The presence of intestinal lesions in certain of the naturally occurring syndromes in man such as pellagra (page 316) and kwashiorkor (page 333) and blacktongue in dogs (page 329) makes careful investigation of the colon an important part of any pathologic study. So far the most conspicuous changes have been found in pantothenic acid-deficient animals (page 228).

of other essential nutrients have not been shown to affect these structures, though it is certain that biochemical observations could be detected with ease.

Ovary: It is difficult to separate the two stages in the development of the ova, that is pre- and post-fertilization As far as the first is concerned, maturation of the ovum is particularly susceptible to inanition. So far no specific alterations have been described to occur as a result of deficiency of single essential nutrients if adequate paired-weight gain controls have been studied. Unfortunately, far too few investigations have been reported which have employed this technique.

During the past decade the effects of intrauterine deficiency states on the embryo have assumed great importance. So far deficiencies of vitamin A, alphatocopherol, riboflavin, folic acid and vitamin B_{12} have been studied. Alterations in a number of areas: cardiovascular system, skeleton, genito-urinary system, et cetera, have been described Their incidence and severity are dependent on the stage of development of the embryo that the deficient state is instituted It is likely that all of these may follow somewhat similar patterns though already modifications may be produced from one to another For instance, riboflavin seems to affect the skeleton in particular and such changes may be modified by calcium and phosphorus deficiency.

Accessory Female Sexual Organs: The uterus, vagina and vulva of vitamin A-deficient animals exhibit the keratinizing metaplasia seen elsewhere. Other changes such as atrophy and alterations in patterns of keratinization of the epithelial cells have been described in any number of deficient states but so far as can be determined all of those are merely the non-specific effects of inanition

RESPIRATORY TRACT

Trachea and Bronchi: A characteristic of the epithelia lining the trachea and bronchi is the presence of cilia These hair-like structures are an important defensive mechanism with respect to inanimate foreign particles and animate agents of disease The replacement of the ciliated columnar cells in the trachea and bronchi of the vitamin A-deficient animal is familiar enough (page 128) It would be interesting to study the physiological activity of cilia from animals deficient in other nutrients *in vitro* since variations in their movement might very well be encountered.

Lungs: No alterations have been encountered in the lungs save those related to mechanical effects locally. Atelectasis as a result of collapse of the thoracic cage is seen in rickets Bronchial obstruction and secondary pulmonary infection follow the keratinizing metaplasia of vitamin A deficiency.

Fatty liver results from choline deficiency, which is in turn related to methionine (protein) deficiency. Cystine makes for increased fat accumulation. So, too, excess fat and cholesterol accentuate fat accumulation. Certain amino acids (threonine, lysine, tryptophan) decrease fat accumulation as does vitamin B_{12}. The whole problem of the relation of fat accumulation to cirrhosis would appear to have been clarified by the recent demonstration that, if the bacterial flora of the intestine is decreased, cirrhosis will not occur. This indicates that all of the various factors will have to be restudied in relation to the presence or absence of a normal bacterial flora.

PANCREAS

Metaplastic alterations in the duct epithelium as a result of vitamin A deficiency occurs in the pancreas (page 126). Experimental inanition and protein deficiency lead to a decrease in the size of the ascinar cells and diminution in the number of zymogen granules. Similar atrophic changes have been described in naturally occurring disease in man, in particular, kwashiorkor (page 340).

GENITO-URINARY TRACT

Kidneys: Clearcut lesions in the nephron have been described by deficiency of each of the following essential nutrients: potassium, magnesium, chlorine, essential fatty acids, and choline. Questionable alterations have been reported as a result of riboflavin and pyridoxine deficiencies. In all of these the tubular portion of the nephron is most seriously affected. The pathogenesis of each is so obscure that little is to be gained by speculating concerning their biochemical backgrounds.

Renal Pelves, Ureter, and Bladder: The epithelial lining of these structures undergoes keratinizing metaplasia as a result of vitamin A deficiency. No alterations have been described in other deficiency states

Testis: The male germinal epithelium is extremely sensitive to inanition. Consequently, whenever growth in general is altered, the testes evince alterations. In some cases this change may be primary; in others it may be modified by administering gonadotrophic hormones. The only deficient state in which specific and irreversible alterations occur is that produced by vitamin E deficiency, though here only the rat and guinea pig are affected. The paired-weight gain technique could profitably be applied to studies of the testis in other deficient states. Most often the paired-feeding procedure has been employed and specific differences have been claimed when such could be just as well ascribed to differences in weight gain.

Accessory Male Sexual Organs: Characteristic metaplasia occurs in the epididymis, prostate, seminal vesicles and coagulating glands in vitamin A-deficient animals. When the effects of inanition are ruled out deficiency

found changes to take place in the adrenal glands as a result of deficiency of any of the nutrients. To date, however, the only vitamin-deficiency state to result in changes is that produced by pantothenic acid. Here extensive necrosis of the cortical cells is seen, this may be made worse by ACTH administration and is retarded or prevented by cortisone.

MESENCHYMAL TISSUES

Connective Tissues. Today there is a growing interest in normal and diseased connective tissues, whether they be ordinary fibrous tissue, cartilage or bone In several places we have noted certain facts concerning the chemical nature of these structures, i e, that they consist of cells surrounded by a matrix whose prominent characteristic is the presence of the fibrous protein, collagen and various mucopolysaccharides, depending on the tissue under discussion [733, 749]

In order to have connective tissue, at least collagen, it is necessary to have its amino acid building blocks Protein deficiency certainly interferes with certain aspects of collagen formation, as exemplified by wound healing A more dramatic effect is seen when ascorbic acid is lacking from the diet of man, primate or guinea pig Evidence begins to point to a role of this vitamin in hydroxyproline synthesis (page 189)

Cartilage and Bone: The growth of cartilage is easily affected by a lack of any one of the essential nutrients This is a non-specific effect, we know of no specific alteration in cartilage which one might pick out and say, "this is due to a deficiency of such and such a nutrient," excepting, of course, the characteristic alterations which are encountered in rickets No deficient state with which we are familiar is associated with increased growth of cartilage cells

Osteoblastic activity may be impaired in an entirely similar manner to that in which cartilage is affected Here, however, two nutrients affect osteoblastic activity far out of proportion to their influence on cartilage cells These are copper and ascorbic acid whose interrelations, if any, would be of interest to elucidate In the classification of bone disease presented in Table XX it will be noted that there is one instance of excessive osteoblastic activity, i e, vitamin A deficiency This is the local bony overgrowth which may lead to certain neurological alterations No examples of decreased destruction are available However, excess destruction may be encountered as a result of deficiencies of calcium and phosphorus, particularly the former. This is not necessarily a parathyroid effect since it is seen in the absence of the parathyroid glands The various causes of rickets in relation to deficiency of calcium, phosphorus and vitamin D have been

ENDOCRINE GLANDS

Hypophysis: Most reports dealing with the response of tissues to nutritional deficiency fail to mention the pituitary gland. The presence of different cell types and the elaboration of a number of trophic hormones might lead one to expect that changes in the hypophysis might be common. So they are as a result of the non-specific effects of caloric restriction, i e., general inanition. When it comes to more specific alterations very little has been reported. "Thyroidectomy cells," i.e., large vacuolated basophils, are seen in animals in which goiter has been produced by iodine deficiency (page 79) One might expect alterations associated with disturbances in function of other endocrine under pituitary control. Such has not yet been reported.

A secondary manifestation of disease is seen in the hypophysis of vitamin A-deficient animals; increased intracranial pressure leads to cysts in the pituitary gland of calves deficient in this vitamin (page 136).

Thyroid: The thyroid gland exhibits hyperplastic changes affected by TSH stimulation whenever the quantity of its own active principle is decreased in circulation. Such situations are summarized in Table IV (page 76) The most important deficiency state is that produced by iodine (page 74) One wonders also if just the right degree of tyrosine deficiency might not be effected by withholding phenylalanine and so lead to insufficient tyrosine with which to form tetra- or tri-iodotyrosine; hence decreased formation of active principle might occur and would be followed by hyperplasia. As has been indicated (page 78), another type of thyroid gland change, colloid goiter, which is such an important form of natural disease, has not been produced in the laboratory.

Parathyroid: The activity of the parathyroid appears to be controlled by the concentrations of calcium in the plasma. Hence, any mechanisms which tend to lower serum calcium, particularly its ionized portion, will lead to parathyroid hyperplasia. Such is seen in calcium deficiency states whether produced by dietary lack, lack of absorption from any means, particularly vitamin D deficiency, and rises in serum phosphorus concentrations. As far as has been determined to date, phosphorus deficiency has not been shown to affect the activity of the parathyroid cells.

Adrenals: Studies of the adrenals have been of particular interest during the past decade since the advent of cortisone and related substances The response of the adrenal cortex to acute caloric deprivation, lack of protein, deficiency of and other nutrients is hyperplasia. This is the "alarm reaction." Changes in the adrenal cells as a result of potassium or sodium deficiencies are of particular interest because of the control of this gland's excretion of the latter cation (pages 31 and 34). One might expect pro-

is not surprising that iron deficiency leads to its disappearance. The relations of tryptophan to the pigment is less clear although this amino acid is, of course, intimately allied to hemoglobin formation. Vitamin A deficiency, of course, leads to changes in the ameloblasts which would explain the dental achromia in this deficiency Absence of the pigment in vitamin E-deficient rats cannot be explained at present. It is of interest that when certain elements, such as cadmium and fluorine, are included in the diet of rats, the yellow pigment does not appear

It was noted above (page 138) that the odontoblasts are organized by the enamel epithelium so that various secondary changes are seen in the dentine as a result of damage to the enamel organ Certain deficiencies, however, primarily affect the physiology of dentine, for instance, vitamin C deprivation leads to a cessation of formation of dentine in conformity with its generalized influence on the elaboration of inter-cellular substances of which dentine is one Defects in the formation of dentine and of the tooth supporting structures are characteristic of the scorbutic state A somewhat different situation prevails in rickets, here the odontoblastic activity is not impaired, but the dentine which is formed is not calcified, because of the disturbance in calcium and phosphorus metabolism Although enamel hypoplasia has not been observed on low phosphorus diets, it has been noted when the calcium intake is restricted

BLOOD-FORMING TISSUES, VESSELS, AND THE COAGULATION MECHANISM

The cells which form the freely circulating red and white blood cells are dependent on a number of nutrients for their integrity Much is known about certain details of erythrogenesis,[1519] less data are available on the mechanisms concerned with the formation of myeloid elements and platelets

Erythropoiesis In the mammalian embryo the earliest stage of red blood cell formation is found in the blood islands of the yolk sac Here, mesenchymal cells give rise to the most primitive erythrogenic cells, to which a variety of names have been applied (hematocytoblast, procrythroblast, et cetera.) Characteristic nuclear and cytoplasmic changes appear in such cells as they develop into adult non-nucleated forms Thus "first generation" or megaloblastic series of cells are encountered in the bone marrow in cases of megaloblastic anemia, whether produced experimentally in animals or occurring naturally in the human

A little later in the development of the embryo, foci of erythropoiesis appear in the liver and, as the cartilagenous skeleton is transformed into

TABLE XX

PATHOLOGIC ALTERATIONS IN CARTILAGE AND BONE AS A RESULT OF DEFICIENCY STATES[517]

I Disturbance in Growth of Cartilage
- (1) Increased Activity
- (2) Decreased Activity
  - (a) Caloric restriction
  - (b) Deficiencies of elements, amino acids or vitamins

II Disturbances in Osteogenic-Osteolytic Balance
- (1) Decreased osteoblastic activity
  - (a) Lack of all essential nutrients, but in particular copper and ascorbic acid and possibly manganese
- (2) Increased osteoblastic activity
  - (a) Vitamin A deficiency
- (3) Decreased osteolytic activity
- (4) Increased osteolytic activity
  - (a) Severe inanition, especially loss of calcium and phosphorus

III Disturbances in the Deposition of Hydroxyapatite in Matrices of Cartilage and/or Bone
- (1) See Tables VII and XII

covered in Tables VII and XII; hence, further discussion is not necessary here.

Teeth: The teeth have not been studied in each of the diseased states which may be produced by deficiency of single essential nutrients. The teeth are more complex than cartilage and bone since they are composed of epithelial cells as well as connective tissue. Hence, they may be discussed from two standpoints depending on which of these two components is initially affected The enamel organ is primarily damaged in both magnesium and vitamin A deficiencies. It is unfortunate that this structure has not been studied more fully in other deficiencies in which ectodermal structures, particularly the skin, are severely involved In magnesium-deficient animals ameloblasts atrophy with the result that the enamel is hypoplastic. Secondary changes occur in the formation of dentine. The enamel organ acts as an organizer of the odontoblasts, and in vitamin A deficiency a lack of this organizing influence is strikingly seen. Because of physiological abnormalities in the ameloblasts, the odontoblasts do not differentiate with a result that the formation of dentine is irregular or absent. The enamel organ in this instance seems to have the same effect on the organization of odontoblasts as does vitamin C.

Interest has also been aroused in teeth because of another functional activity of the ameloblasts: the formation of the familiar yellow, iron containing pigment of the rat's incisor. Failure of this pigment to be deposited has been noted to result from deficiency of several nutrients: iron, tryptophan, vitamin A, and alpha-tocopherol. Since this material contains iron it

type of megaloblastic red blood cell formation has not been satisfactorily explained. Copper is a factor in erythropoiesis, as has been indicated on page 57.

The basophilic ascribed to nucleoprotein in the cytoplasm of the erythroblast diminishes as the cells mature, eosinophilic, indicative of hemoglobin formation, appears. A good deal is known of many of the mechanisms concerning the synthesis of the pigment, heme, and the protein, globin Heme is a combination of protoporphyrin and iron The former is derived from glycine via an intermediate, amino-levulinic acid Since the Krebs cycle is involved in this transformation, one would expect pantothenic acid to play an important role, anemia has been observed as a result of a deficiency of this vitamin. So, too, folacin appears to be implicated in protoporphyrin synthesis. Pyridoxine affects protoporphyrin metabolism, since a decrease in erythrocyte free protoporphyrin is found in animals deficient in this vitamin Copper appears to be necessary for the incorporation of iron into the protoporphyrin molecule to form heme (page 59) The importance of copper for iron absorption must also be recalled (page 59)

Many amino acids are necessary for the synthesis of globin Furthermore, histidine appears to be important in the combination of globin with heme. Some amino acids are more potent than others in correcting the hemoglobin deficit following blood loss.[910]

Another factor which must be considered in any discussion of erythropoiesis and nutritional anemia is the life span of the red blood cell Cells which are formed under adverse circumstances and which might be expected to have a reduction of certain intracellular essential nutrients might be expected to be more liable to destruction Such is the case with respect to the microcytes which appear in the circulation of copper-deficient swine (page 58) In pernicious anemia the life span of the circulating cells is also decreased [1418]

Certain aspects of the role of essential nutrients in red blood cell formation are summarized in Table XXI and in Figure 181

Myelopoiesis: Not much attention has been directed at disturbances associated with the granulocytic series which may result from deficiency states Leukopenia was described in the panmyelocytopenia syndrome which led to the discovery of folic acid The other changes which have been described are less spectacular and need further study.

Lymphopoiesis: The specific tissue of lymph nodes and spleen which forms the lymphoid cells is very susceptible to inanition and protein deficiency. A relation of lymphoid tissue to pyridoxine has been suggested (page 245).

Blood Vessels: Whether any nutrient specifically affects the proliferation of endothelial cells has not been definitely shown. Dr. Wolbach

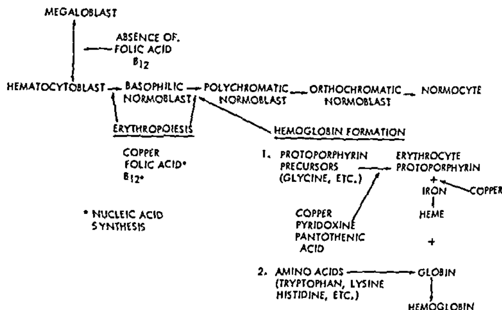


FIGURE 181.

a bony one with marrow spaces, similar changes here occur also The proliferation of erythroid elements at these sites represents the "second generation," or the normoblastic phase of differentiation which is seen normally throughout the remainder of the life of the organism. Many important biochemical transformations occur in cells exhibiting the various stages of megaloblastic or erythroblastic erythropoiesis Of these, the most important deal with nucleoprotein and hemoglobin synthesis, though the formation of complex polysaccharides and lipids must not be forgotten, even though less is known about them from the nutritional standpoint.[1519]

Of the nucleoproteins, desoxyribosenucleic acids are found in the nucleus; ribosenucleic acids are restricted to the nucleolus and cytoplasm. One of the recent contributions to nutritional biochemistry is evidence showing the relation of folacin and vitamin B_{12} to nucleic acid synthesis We cannot go into the details of the classical transformations: purines and pyrinidines→nucleosides→nucleotides→nucleoproteins. However, it will be recalled (page 272) that folacin has been shown to effect the incorporation of 1-carbon fragments into the purine ring. So, too, vitamin B_{12} appears to influence the transformation of purines and pyrinidines into nucleosides. The "maturation effect" of folacin and vitamin B_{12} is obscure. Why the usual normoblastic phase of erythropoiesis reverts back to the embryonic

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Blood Vessels: Whether any nutrient specifically affects the proliferation of endothelial cells has not been definitely shown. Dr. Wolbach

BLOOD AND TISSUE CHANGES IN NUTRITIONAL ANEMIAS

NUTRIENT	TYPE OF ANEMIA	BONE MARROW HYPERPLASIA	HEMOSIDEROSIS LIV., SPL., BM	ERYTHROCYTE PROTOPROPH.	PLASMA IRON	PLASMA COPPER	LEUKOPENIA	N. S. INVOLVEMENT
COPPER	MICROCYTIC HYPOCHROMIC	+	0	N	LOW	LOW	MOD.	+
IRON	MICROCYTIC HYPOCHROMIC	+	0	N	LOW	SL. ELEV.	NO	0
TRYPTOPHAN	NORMOCYTIC NORMOCHROMIC	+	0	N	N		YES	0
PROTEIN	NORMOCYTIC NORMOCHROMIC	+	0	N	N	LOW	NO	0
RIBOFLAVIN	NORMOCYTIC NORMOCHROMIC	+	0				NO	+?
PYRIDOXINE	MICROCYTIC HYPERCHROMIC	+	+	LOW	HIGH		NO	+
FOLACIN	MICROCYTIC HYPERCHROMIC	+ MEG.	+	LOW	HIGH	N	+	
B_{12}	MICROCYTIC HYPERCHROMIC	+ MEG.	+		HIGH		+	+
PANTOTHENIC ACID	NORMOCYTIC NORMOCHROMIC	+	0				NO	+

maintained that capillaries cannot form, i.e., organizing a hematoma, when ascorbic acid is lacking. Our own studies of scorbutic animals in which the invasion of cartilage was unimpaired seemed to controvert this. A pointed study of this needs to be undertaken. The studies which relate the capillary bleeding in scurvy to alterations in the precapillary vessels are most interesting (page 193). The whole problem of the maintenance of the integrity of the capillary wall is most complex and until more is known of the normal state advances will be slow in understanding the abnormal.

Coagulation Mechanism: The subject of the clotting of blood becomes more complex each year and, hence, more difficult for the bystander to comprehend. It is significant that deficiency states, that is inborn defects, have been responsible for bringing at least some complex order out of chaos A simplified scheme of the coagulation mechanism is presented in Figure 182 where the factors which may affect the various steps are indicated [1520, 1521, 1522]

Here it will be seen that calcium assumes great importance.[1523, 1524, 1525] So, too, vitamin K is responsible for the formation of at least three factors which have to do with coagulation. Anything which may interfere with

A SIMPLIFIED SCHEME OF BLOOD COAGULATION

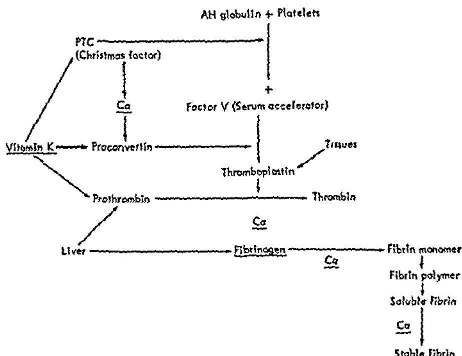


FIGURE 182

protein synthesis such as amino acid deficiency or liver disease will lead to defects in the coagulation mechanism

Clinical aspects of the relation of vitamin K to coagulation have been discussed by Dam [2328]

Finally, although the platelets are of importance in coagulation, no deficiency states are characterized by extensive changes in thrombocytes, save perhaps iron deficiency in which the platelets are increased (page 65).

MUSCLE TISSUES

Heart. The myocardium is dependent on an abundant supply of oxygen which must be furnished by an adequate flow of red blood cells containing a normal quantity of hemoglobin In addition, certain substrates are necessary to furnish energy, particularly that for muscular contraction. Studies

on the human, utilizing techniques to obtain blood samples from the coronary vein, femoral artery, pulmonary artery, et cetera, have shown that the myocardium removes appreciable amounts of lactate, pyruvate, and glucose.[1526] Normally, too, though to a lesser degree, nitrogenous sources such as amino acids, fatty acids and ketones may be used as substrates, as may be evinced by their extraction from arterial blood supplying the myocardium.

Certain of the elements: sodium (page 32); potassium (page 24) and calcium (page 43) were shown by Sidney Ringer many years ago to be indispensable for the normal functional activity of the heart. Magnesium does not appear to be so important. The role of many indispensable ions as integral parts of certain enzymes and as activators of certain enzymes has been mentioned elsewhere (page 19). The importance of magnesium in this respect has been commented upon (page 35). So, too, the possible role of copper in affecting the cytochrome oxidase activity of the myocardium has been mentioned (page 64). The effects of deficiency of the other essential inorganic elements on the myocardial fibers are not clear. One might theorize that iodine deficiency might ultimately lead to the production of a myxedematous change in the heart such as is seen in the human hypothyroid state. Such has not been produced experimentally. So, too, iron deficiency might be expected to produce myocardial changes because of the presence of this element in the myoglobin molecule. This does not appear to happen because myoglobin gets the "first call" on the iron. The effects of anemia on the heart are well-recognized.

Of all the inorganic elements, a deficiency of only one, potassium, is associated with structural defects in the myocardium. Such alterations consist of necrosis of individual fibers The mechanism underlying this alteration is unknown.

Some evidence has been presented that a deficiency in at least one amino acid, tryptophan, may lead to changes in the myocardium (page 91).

In view of the constant contraction of the myocardium, for instance, 112,320 times each twenty-four hours, the energy needs of the heart are great. The myocardium is dependent on a large number of enzymes for its aerobic metabolic requirements Many of these enzyme systems require coenzymes containing certain vitamins for their activity. Physiological alterations which have been observed in vitamin deficiency states are summarized in Table XXII.[1527] From what has been noted elsewhere concerning the normal functions of the vitamins (Figure 45, page 124), these deranged functions in the myocardium are what might be expected.

So far only two vitamins, thiamine and alpha-tocopherol, have been shown to lead in morphological changes when their intake or utilization

TABLE XXII

BIOCHEMICAL ALTERATIONS IN THE MYOCARDIUM IN VITAMIN DEFICIENCY STATES

Vitamin	*Biochemical Defect*
Thiamine	Decreased cocarboxylase content, increased pyruvate content
Pantothenic Acid	Reduced CoA, oxygen uptake, and pyruvate utilization
Pyridoxine	Decreased transaminase activity
Niacin	Decreased utilization of lactate
Biotin	Decreased oxygen uptake

is interfered with The lesions, which consist of necrosis of the myocardial fibers, are identical save that ceroid is seen in the latter group. More pointed study of the heart muscle in other deficient states may lead to an unearthing of morphological alterations in these also The role of choline deficiency needs further study (page 261)

An extremely interesting group of anatomical defects has been produced in the cardiovascular system of the rat as a result of intrauterine vitamin deficiency. Four nutrients have so far been implicated· riboflavin (page 216), folacin (page 275), vitamin B_{12} (page 279) and vitamin A (page 139) The lesions, which can be made to appear during certain important stages of development of the cardiovascular system, affect the myocardium and, in particular, the large arterial trunks arising from the heart The experimental production of congenital cardiac malformations would appear to be a fertile field for future development. Whether more than *one* basic pattern of derangements may be produced remains to be determined

Striated Muscle: Unlike the heart, striated muscle may metabolize aerobically and anaerobically Hence, its biochemical transformations are *more complex* Most of *the basic requirements for energy* are furnished by glucose and lactic acid For their metabolism it needs certain elements, amino acids and vitamins Defects in the contractile mechanism of striated muscle have been described as a result of decreased serum concentrations of three inorganic elements. potassium, calcium and magnesium, which play such an important role in the tetany syndrome (page 295). In only one of these deficient states, that produced by potassium, are structural defects encountered. Necroses have been observed in several species deficient in this cation

Despite the undoubted need for other nutrients in the normal metabolic functions of striated muscle, relatively few data are available to indicate *biochemical or structural* changes *resulting from* deficiencies of more than a few Dystrophic alterations have been observed to result from deficiency of the *amino acids, tryptophan and isoleucine* (pages 91 and 95), and from lack of vitamin E (page 163).

Studies of nutritional muscular dystrophy are, *of course, of great in-*

terest because the lesions are identical with those which are seen in many instances of human disease. Unfortunately no relationship to dietary factors has been shown in the human muscular dystrophies.

Involuntary Muscle: In Mammalia, lesions have been described, though inadequately, in the smooth muscle of rats deficient in tryptophan. Pigment, of course, occurs in smooth muscle of vitamin E-deficient animals, the significance of this deposit is not clear even at this time.

NERVOUS TISSUES

In the discussions of alterations resulting from deficiencies of single or multiple nutrients, disturbances in the central and peripheral nervous systems have figured prominently. Moreover, it is likely that, if more careful studies were made, further physiological and anatomical defects might be uncovered.

In discussing the nervous tissues we are, of course, concerned with the metabolism of carbohydrate, protein and lipid.[1506] The first two materials doubtless are of most importance to the activity of neurons while the latter is concerned in the formation of myelin, which is a complex lipid material. Hence, this brief résumé of the nervous tissues might most advantageously be directed towards a summary of the derangements which accompany defective metabolism of carbohydrates, proteins and fats.

The importance of adequate glucose for the normal activity of nervous tissue is clearly shown by the physiological effects of hypoglycemia, however produced (page 118). If the hypoglycemia is prolonged, structural defects may be found in addition to the functional alterations which precede them. Since nervous tissue is so dependent on carbohydrate for its integrity, essential nutrients which have to do with the metabolism of glucose and its transformation to pyruvate and acetate would be expected to lead to similar changes. The most prominent example of this is, of course, thiamine deficiency. Here *in vitro* studies of respiring brain tissue led Peters to the description of a biochemical lesion which he showed to be an inability to metabolize pyruvate[757] When thiamine was added to the *in vitro* system, the pyruvate content fell. These experiments aided greatly in clarifying the derangements in thiamine-deficient animals in which structural lesions are frequently conspicuous by their absence. It is likely that, when the deficiency of thiamine has continued for some time, morphologic defects will appear in the form of necrosis of neurons followed by reparative proliferation of glial cells and blood vessels. Whether this represents the pathogenesis of the Wernicke syndrome remains to be determined. An important point to settle is the primary or secondary status of the vas-

cular system in thiamine-deficient animals (and in Wernicke's disease). One suspects that the capillary change is a secondary phenomenon. Lipoic acid deficiency, if it can be produced, should lead to the same alterations as does deprivation of thiamine (page 205)

Neuronal damage is encountered when the nicotinic acid antagonist, 3 acetyl-pyridine, is administered Although magnesium is extremely important in the metabolism of carbohydrate, it appears that levels low enough for it to interfere with the breakdown of glucose and its various intermediates cannot be attained (page 41). Other vitamins which might be expected to be involved in carbohydrate metabolism are pantothenic acid and riboflavin Structural alterations have been described in pantothenic acid-deficient swine and other species In the pig only the sensory neuron has been shown definitely to be involved. Our own interpretation of this alteration is that the neuron body is damaged first, followed by secondary changes in its axoplasm and myelin sheath This conclusion was drawn because the first alteration which could be detected was chromatolysis, a change which preceded any myelin degeneration Riboflavin deficiency leads to alterations in the nervous tissues of some animals. The data are too fragmentary at the present time to warrant further discussion

The precise role of vitamin B_{12} in the pathogenesis of the alterations in the nervous tissues in pernicious anemia is not yet satisfactorily settled That is, what is the phase of metabolism which is basically deranged that leads to the functional and structural disturbances? It is not clear whether the structural alterations in pernicious anemia fall into the polioclastic or myelinoclastic group of diseases Is the primary disturbance one of carbohydrate metabolism, nucleic acid synthesis or myelin synthesis and maintenance? After all these years the primary or initial neurological disturbance in pernicious anemia is still a mystery Perhaps future experiments with vitamin B_{12} antagonists may throw some light on this subject. In this respect some experiments recently reported in preliminary form by Vogel[1091] are extremely important When the peripheral nerves of animals on various deficient diets were traumatized, the regenerative capacity was affected particularly by lack of pyridoxine and vitamin B_{12} In the latter groups regeneration of the axoplasm was not impaired, while myelin production failed to keep pace On the other hand, pyridoxine deficiency led to a decrease of both axis cylinder and myelin repair These data begin to indicate that vitamin B_{12} deficiency leads primarily to a disturbance in myelin metabolism and, hence, the Schwann cells, which appear to be responsible for myelin formation, are primarily defective We shall have to await further studies in order to have the entire story

With respect to myelin metabolism, some years ago we suggested that the specific defect in pyridoxine deficiency might be a disturbance in the

terest because the lesions are identical with those which are seen in many instances of human disease. Unfortunately no relationship to dietary factors has been shown in the human muscular dystrophies.

Involuntary Muscle: In Mammalia, lesions have been described, though inadequately, in the smooth muscle of rats deficient in tryptophan. Pigment, of course, occurs in smooth muscle of vitamin E-deficient animals; the significance of this deposit is not clear even at this time.

NERVOUS TISSUES

In the discussions of alterations resulting from deficiencies of single or multiple nutrients, disturbances in the central and peripheral nervous systems have figured prominently. Moreover, it is likely that, if more careful studies were made, further physiological and anatomical defects might be uncovered.

In discussing the nervous tissues we are, of course, concerned with the *metabolism of carbohydrate, protein and lipid.*[1506] *The first two materials* doubtless are of most importance to the activity of neurons while the latter is concerned in the formation of myelin, which is a complex lipid material. Hence, this brief résumé of the nervous tissues might most advantageously be directed towards a summary of the derangements which accompany defective metabolism of carbohydrates, proteins and fats.

The importance of adequate glucose for the normal activity of nervous tissue is clearly shown by the physiological effects of hypoglycemia, however produced (page 118). If the hypoglycemia is prolonged, structural defects may be found in addition to the functional alterations which precede them. Since nervous tissue is so dependent on carbohydrate for its integrity, essential nutrients which have to do with the metabolism of glucose and its transformation to pyruvate and acetate would be expected to lead to similar changes. The most prominent example of this is, of course, thiamine deficiency. Here *in vitro* studies of respiring brain tissue led Peters to the description of a biochemical lesion which he showed to be an inability to metabolize pyruvate.[737] When thiamine was added to the *in vitro* system, the pyruvate content fell. These experiments aided greatly in clarifying the derangements in thiamine-deficient animals in which structural lesions are frequently conspicuous by their absence. It is likely that, when the deficiency of thiamine has continued for some time, morphologic defects will appear in the form of necrosis of neurons followed by reparative proliferation of glial cells and blood vessels. Whether this represents the pathogenesis of the Wernicke syndrome remains to be determined. *An important point to settle is the primary or secondary status of the vas-*

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With respect to myelin metabolism, some years ago we suggested that the specific defect in pyridoxine deficiency might be a disturbance in the

metabolism of this material. This was based on a comparison of the lesions due to single deficiencies of pantothenic acid or pyridoxine. The primary alteration associated with the latter deficiency was myelin degeneration which occurred in the absence of neuronal change which is such a prominent reaction to pantothenic acid deficiency. These studies have never been continued and certainly should. The studies reported by Swank and Adams [1513] on material from the Merck Institute are not comparable since they did not have an opportunity to investigate changes in animals dying early in the course of the two deficiencies

Pyridoxine deficiency leads to convulsions in various species including man. Exactly what metabolic disturbance is responsible for these fits is not clear. The role of pyridoxine in protein metabolism might indicate a disturbance in glutamic acid metabolism or in the conversion of tryptophan to serotonin.[1506]

The participation of at least two elements, calcium and magnesium, in cerebral neurological activity would appear to be demonstrated by the fits which occur when either one of these cations is removed from the diet of experimental animals. Similar convulsive seizures are seen in pyridoxine-deficient animals and have also been encountered clinically in infancy.

The story of endemic copper deficiency and the damage to the brain which ensues has been told. It is unfortunate that similar neurological lesions have not been produced with ease so that they could be intensively studied in the laboratory. A similar gap is present in our understanding of the precise pathogenesis of the neurological alterations in pernicious anemia Here, perhaps, the synthesis of an anti-vitamin B_{12} may allow us to produce lesions in spinal cord and brain similar to those seen in clinical deficiency states.

Two other basic pathologic alterations deserve mention. The first concerns blood vessels and is, of course, concerned with their integrity. These are the hemorrhages encountered in vitamin K-deficient animals. The second involves the dissimilarity in growth of the brain and spinal cord and their bony coverings which is seen in the presence of deficiency of vitamin A. As a result of slowing of development of the latter tissue the brain and cord herniate through various foramina and mechanical defects are produced.

The exact pathogenesis of the naturally occurring neurological syndromes which have been described in association with dietary deficiencies will doubtless never be worked out. The many variable factors other than those imposed by lack of nutrients themselves make clarification a near impossible task. The reader will find an abundance of material in the bibliography, and he will doubtless agree that the situation is extraordinarily confused.[1287, 1288, 1457, 1490]

Part IX

Deficiency Disease as a Research Method in Biology and Medicine

The fully developed concept that disease could result from deficiency of certain essential nutrients had to await the discovery of the nutrients themselves. Lind's[700] observations on the role of fruit juices in the prevention of scurvy and Magendie's experiments on the inadequacy of certain foodstuffs were a beginning Not until the latter half of the 19th century did the relationship of nutrition to disease begin to assume any importance In 1878 Wernich wrote concerning pernicious anemia, "Like beriberi, scorbutus, chlorosis, etc., it belongs to a class of constitutional diseases brought about by disturbances in nutrition"[1393] Just at this time, however, which was also when Lunin had begun his experiments with simple diets, all attention was centered on the role of animate agents in the causation of disease. Bacteria and their poisons were indicted in the pathogenesis of many human diseases; this detracted from studies relating certain syndromes to deficiency states Even by 1913, when Funk had elaborated his thesis concerning the importance of certain dietary factors which he called "vitamines"[469] and Vedder had published his monograph on beriberi,[1352] the concept of deficiency disease was too new for most to grasp This was clearly brought out by an often-quoted statement in the British Medical Research Council's 1924 *Report on the Present State of Knowledge of Accessory Food Factors (Vitamins)* "Disease is so generally associated with positive agents—the parasite, the toxin, the *materies* morbi—that the thought of the pathologist turns naturally to such

accomplished. So, too, a prophesy which Dr. Wolbach made in his DeLamar Lecture of 1937 has begun to reach fruition. He said, "The steady progress in understanding of the biochemistries of the vitamins now obtainable in pure form is a challenge to the cytologist because in some instances it should be possible to determine the loci, within cells, of vitamin activities. The opportunity of associating chemical activities or functional roles within nuclear and cytoplasmic structures appears to be at hand"[1494]

At the same time, others had also realized that a new era in biochemistry had been reached For instance, Peters stated in 1936, "We are so accustomed to the detailed analysis upon the fixed tissue which is made possible by refined histological methods that we do not readily adjust to the idea that a new type of analysis is being steadily perfected by modern biochemical research. So far as the separation of one cell from another is concerned and the elucidation of differences in its pathological state, biochemistry is

still very crude. We cannot work upon much less than 50 mg. of wet tissue, but we can obtain information from this of changes too subtle to be revealed upon the fixed histological specimen, changes in the behavior of essential enzymes systems present. It really constitutes a new approach to pathological analysis."[757]

As the above words were spoken, Bensley and Hoerr had already begun their studies on the fractional centrifugalization of cellular constituents, which Claude, Schneider and Hogeboom were to develop and which have so profoundly influenced present-day biochemistry.[1495]

Now the electron microscope has been added to other tools with which one can study the structure of cells. This permits the biochemist to be even more precise in his understanding of the fine structure of the materials which he is investigating, and has opened up an entirely new area in morphology.

The present situation can be summarized in part by the following sentences "The history of medicine teaches us, if we will only take a somewhat comprehensive survey of it, that at all times permanent advances have been marked by anatomical innovations, and that every more important epoch has been directly ushered in by a series of important discoveries concerning the structure of the body. So it was in those old times when the observations of the Alexandrian school, based for the first time upon the anatomy of man, prepared the way for system of Galen; so it was, too, in the Middle Ages, when Vesalius laid the foundations of anatomy, and therewith began the real reformation of medicine; so lastly, was it at the commencement of this century when Bichat developed the principles of general anatomy."

These words were spoken 100 years ago by Rudolph Virchow in the first of his lectures on *Cellular Pathology* which were delivered during the winter of 1858.[1496] If we were to add a brief summary of the knowledge which was gained during the past century, together with the current advances which have been made in cytochemistry and submicroscopic anatomy, Virchow's words would be entirely applicable now. Moreover, pathologists are in the same position today as he indicated them to be in 1858, "what Schwann, however, has done for histology, has as yet but in a very slight degree built up for pathology."[1496]

Pathologists are once more the recipients of "anatomical innovations." The use of these techniques for the study of normal structure and function should allow us to proceed to unravel further some of the complexities of abnormal states, one of which is deficiency disease.

From the material which has been presented in the preceding sections it must be obvious that much information concerning the pathogenesis of deficiency disease has already been collected. In the process new facts have been obtained which contribute to our understanding of the normal

structure and function of tissues. One cannot ever study disease without learning something of the normal. Yet all we have related, frequently too briefly, is only a beginning There is now need for those trained in other disciplines to direct their attentions to the many unanswered problems which confront the student of deficiency disease Such an interdisciplinary approach should be fairly obvious Yet it might not be amiss to mention some of the specialties which might be implicated and to point out in general paths which further investigations might take

The general *histologist* has already profited greatly from the studies of controlled collagen formation, spermatogenesis, erythropoiesis, et cetera He is now in a position to apply an increasing number of histochemical reactions to normal and diseased tissues Moreover, he has at his command techniques which have been developed by the biophysicist, such as electron microscopy, microradiography, autoradiography, phase contrast techniques, polarization optics, and interference microscopy

New methods such as those developed by Eagle for growing cells in chemically defined media, should allow the *cytologist* to study intimate changes resulting from deficiencies of single elements,[16] amino acids[405] and vitamins.[1507]

The *embryologist* and *obstetrician* have already obtained important data on reproductive performance and the incidence of congenital malformations as influenced by deficiencies of various nutrients

The *geneticist* has had little to do with deficiency disease yet here much can be done. Already two diseases, rickets and dental caries, have been shown to be modified by hereditary factors. Other deficiency states may doubtless be shown to exhibit similar patterns

The *endocrinologist* has already benefited from studies of the effects of deficiencies on each of the endocrine organs. The relations of iodine to goiter, of calcium deprivation to the parathyroid, of sodium and potassium deficiencies to the adrenal, et cetera, are just beginnings and should lead to more information in the future

The *microbiologist* has studied the reactions of the deficient host to various animate agents of disease for many years So, too, *virologists* and *parasitologists* have taken up this phase of investigation The intimate relationships between parasite and host cell, which can now be visualized with the electron microscope, should stimulate studies utilizing deficient states. The use of germ-free animals is another extremely important technique to cultivate.[1514] The role of the intestinal flora in ruminants and non-ruminants has received much attention[1515] Will not all of the studies of essential nutrients have to be repeated on germ-free guinea pigs, rats, and other species?

The *immunologist* has already utilized and should continue to employ

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deficiency states with which to study antibody response. Studies on hypersensitive reactions and hemolytic phenomena have scarcely begun.

Another specialty which is assuming increasing attention is *geriatrics*. Already long term studies such as those of McCay and others have yielded important information.[1516, 1517] Histological examination of animals whose lives have been shortened or lengthened are much to be desired.

Analyses of nutritional diseases which occur naturally have already appealed to the *epidemiologist* and *medical ecologist*.[1518] Pellagra could be cited as case in point. Its geographical distribution and relation to maize was of importance in an understanding of its pathogenesis. So, too, its local distribution made it clear at once to Goldberger, who was an accomplished epidemiologist, that the disease was not of an infectious nature. The epidemiological approach to rickets, endemic goiter, beriberi and kwashiorkor has led to a much better understanding of these syndromes.

As an adjunct to cancer research deficiency disease has opened new approaches for the *oncologist*, though as yet the results have been of more value to nutrition than to oncology.

For the *comparative pathologist* and *veterinarian* the field of deficiency disease should be of particular interest. Studies of both naturally occurring as well as experimentally produced deficiency disease have been most revealing to the biochemist and professional nutritionist. Mineral deficiencies in the field, vitamin deficiencies in captive animals, experimental studies in small laboratory animals, all need investigation by this group.

These general comments may indicate some of the ramifications of research in deficiency states. Obviously, the most important domains continue to be the fields of biochemistry and pathology, or, if one cares to look into the future and will pardon the terminology, the field of cytopathobiological chemistry. The pathogenesis of disease always comes down to a question of too much or too little. The latter is what makes studies of deficiency disease so fundamental For interference with the nutrition of the cell is the fundamental basis of all disease, whether one is concerned with oxygen supply, removal of carbon dioxide, acidosis, alkalosis, accumulation or loss of any number of exogenous or endogenous nutrients, et cetera. At some future time, perhaps, when more data are at hand, such an etiological approach can be employed in the preparation of a monograph dealing with the pathogenesis of virtually all disease.

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